SOME ASPECTS OF BOVINE RETAINED PLACENTAE

by

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The diagnosis, treatment and prevention of disease of the bovine reproductive tract are major problems confronting the large animal veterinary practitioner of today. The realization of the importance of dairy produce in human nutrition, together with the development of methods of distribution of fresh milk and beef to the urban consumer, has resulted in a markedly increased popularity of these products until their production has become a major industry in the United States. Elimination of foot and mouth disease, bovine tuberculosis and Texas fever as great animal plagues has left brucellosis, mastitis and sterility as problems of prime concern to the modern practitioner of bovine medicine. Of these brucellosis and mastitis are under constant consideration in practically every animal research station in the nation. As to sterility, the literature gives but little definite information for the handling of those abnormalities of the bovine reproductive tract that result in lowered fertility or complete sterility. The problem of sterility must rank close to mastitis, and brucellosis as a cause of economic loss to the dairy and beef industries. Authentic data regarding this question seem to be lacking. The losses in calf crop, milk production which normally can only follow reproduction, and from females sent to slaughter because of failure to breed are enormous.

The loss is greatest among dairy cattle, most of which die from, or are discarded because of genital disease or mammary affections without having produced the expected number of healthy

young. The most informative data found on the subject was recorded by Wing (1933) concerning the dairy herd at Cornell University from 1889 to 1928 inclusive. During the 40 years covered in his report the herd averaged 3.35 pregnancies per cow with an average of 2.4 viable calves per cow. In this herd, where competent veterinary service and excellent herd management were available at all times, this seemingly poor record is undoubtedly better than that of the average large commercial herd. Unofficial information from competent veterinarians and herdsmen lead one to suspect that the actual average reproductive record of the American dairy cow is much less than the average of 3.35 pregnancies per cow recorded by Wing in the Cornell herd.

Among the common causes of sterility in cattle are functional abnormalities of the ovaries, uterine infections, retained placentae and associated metritis and pyometra. While much has been written on these subjects, there are still many gaps in our information. A surprising lack of critical research on the proper clinical management of these pathological conditions is evident to anyone who has reviewed the literature. Most of the therapeutic measures advocated for their alleviation are empiric.

It is always difficult to measure the value of any therapeutic measure. This is particularly true in bovine genital disease. The high cost of experimental animals, their slow rate of reproduction and the expensive equipment necessary to maintain them precludes formal controlled experimentation. Any attempt to evaluate therapeutic measures in this field must rely on clinical

observations on animals over which the clinician does not always have complete jurisdiction. Clients seldom agree to experimentation on their valuable livestock, and all too frequently the clinician finds himself unable to follow the record of animals he has treated whose histories would be revealing.

Because of his association with the veterinary clinic of Kansas State College, the author has had the opportunity to treat the genital disease problems of several dairy herds in which he was able to make repeated examinations of animals that had been treated and to carry on a limited amount of experimentation.

The purpose of this paper is to measure the therapeutic effects of stilbestrol on retained placentae, and pyometra following retained placentae, and to study the blood cell picture in bovines with retained placentae.

REVIEW OF THE LITERATURE

Dodds and his co-workers in 1938 announced the synthesis of a compound, 4:4'-dihydroxystilbene, and, in view of the fact that it was the mother substance of natural estrogenic agents, suggested the name stilbestrol. They pointed out that the substance could be readily prepared in the laboratory at a reasonable cost, and that it possessed estrogenic properties two or three times as great as natural estrone.

Stilbestrol became available for experimental use in America in 1939, and appeared on the market in 1940. Since that time much interest has been shown in its possible clinical uses, and it has been the subject of considerable investigation by many workers.

That stilbestrol has an effect similar to estrone on the mammary gland was reported by Lewis and Turner in 1940 in experiments on dry, lactating and virgin cows. They found it capable of stimulating growth of the mammary ducts and, to less extent, of lobular-alveolar tissue in immature virgin heifers. Prolonged administration in some instances induced lactation in virgin and dry animals, but did not consistently augment milk flow in previously lactating animals.

In 1941 these same workers reported more extensive studies of the mammary effect. They found that relatively small doses stimulated mammary growth in spayed female and entire male mice, castrated male rats, entire male rabbits and virgin goats. Subcutaneous injection in female rabbits followed by treatment with lactogenic hormone induced lactation. The drug did not augment mammary development in a castrated male goat, although there was some teat hypertrophy.

Again in 1942 Lewis and Turner reported that stilbestrol established copious and prolonged lactation in virgin and dry goats whether injected in doses of 0.25 mg. per day, given orally 5 mg. per day, or administered by inunction. This they considered significant because of the ability of the estrogens to increase the anterior pituitary lactogenic output. They found that anterior pituitary extract was effective in augmenting the production caused by stilbestrol by supplementing the increased lactogen output with other anterior pituitary factors, or in initiating lactation in stilbestrol treated goats. Stopping daily treatment was sometimes effective in improving milk production for a time once

it had been inaugurated, but restarting treatment had little effect. Production of normal parturient goats was not benefited by hormone treatment. With one exception the ovaries were essentially normal after long periods of treatment. Two goats were bred out of season after termination of stilbestrol injections, and one bore a kid. No adverse effect on growth or health of any of the goats was observed as a result of stilbestrol treatment.

Folley (1941) stated that estrogens are capable of stimulating the flow of a rich milk, when given in small doses. The effect manifests itself as an increase in fatty and non-fatty solids in the milk. The threshold for the enrichment effect is lower than that of the inhibition effect, which has been repeatedly reported. In general, small doses cause enrichment while large doses cause inhibition. The two effects seem more readily separable with natural estrogens than with diethylstilbestrol.

Results of a series of experiments on the enrichment of cow's milk by the administration of diethylstilbestrol were reported by Folley, Watson and Bottomley (1941). They found that diethylstilbestrol orally did not affect the composition or yield of milk, but the subcutaneous injection of an aqueous solution or subcutaneous implantation of the crystals increased the percentage of milk solids in Shorthorns without decreasing milk yield. In Ayrshires the percentage of milk solids was increased but the yield declined following subcutaneous injection. Diethylstilbestrol dipropionate either in oily solution or in the form of an ointment sometimes increased the percentage of milk solids. In some cases the increase was accompanied by a decrease in yield. Following

large doses of the dipropionate by inunction two out of four Holsteins in advanced pregnancy aborted. Treated cows, especially if treated more than once, were difficult to get in calf subsequently. The enrichment of the milk represented an actual increase in solids, not merely an increased concentration due to reduced secretion of water.

Folley and Young (1941) subjected two multiparous goats, which had been dry for some months after having been brought into artificial lactation with diethylstilbestrol during the previous year, to three times weekly inunction of the udder with 1.0 gm of one per cent diethylstilbestrol ointment. In one case lactation quickly set in and the yield rose to a maximum of 570 cc daily, and then began to decline. At this point subcutaneous injection on alternate days of anterior pituitary extract was begun and the inunctions continued. The milk yield rapidly rose to about 870 cc daily, and later declined somewhat in spite of continued treatment. The other goat secreted no more than 20 cc of milk daily during the period of inunction alone, even when the dose of diethylstilbestrol was doubled for a time, but the addition of anterior pituitary extract injections to the treatment immediately evoked copious milk secretion, the daily yield rapidly rising to nearly 1700 cc. Chemical analyses showed that the milk secreted by both goats was normal. The authors concluded that combined treatment with diethylstilbestrol and anterior pituitary extract could produce a much more intense lactation than could diethylstilbestrol alone. It seemed likely to them that under suitable conditions the artificial lactation so evoked might rival that normally following parturition.

The absorption from tablets of diethylstilbestrol and hexestrol subcutaneously implanted in cows was found by Folley (1942) to fall off at a greater rate than could be accounted for by the decrease in the surface area of the tablet. He found that tablets which had been removed from cows appeared to be insoluble in alcohol or ether, but estrogen was gradually extracted and the tablet began to float. The name "ghost" was proposed for the hollow shell of the original tablet, composed of ether-alcohol insoluble material and evidently formed in vivo by infiltration into and deposition of tissue protein in the pores of the surface zone of the tablet. The presence of this shell could not be detected until the estrogen had been dissolved away. Failure to allow for the weight of the ghost may, according to Folley, amount to errors of from 10 to 20 per cent in determining absorption rates. Infiltration of the cortex of the tablet with so insoluble a substance may considerably retard absorption, and perhaps ultimately stop it completely.

Walker and Stanley (1941) studying the effect of diethylstilbestrol dipropionate on mammary development and lactation, found that estrogenic substances injected into castrate heifers would induce mammary development and copious milk secretion. The turgidity of the udder may be used as an indicator of the secretory activity of the nonlactating gland according to these authors. They found that the titer of estrogen determines its effect on the mammary gland. A low titer induced proliferation of the parenchyma and secretion of milk, while a high titer suppressed lactation, and brought on involution of the mammary gland.

Crafts (1941) studied the effects of estrogens on the formed elements of blood in dogs and rhesus monkeys. He found that daily injections of 5 to 10 mg of stilbestrol in dogs caused a marked neutrophilia followed by a neutropenia, and an increase followed by a decrease in young neutrophils, and anemia, and a decrease in hemoglobin. Similar results were obtained by daily doses of estradiol. In rhesus monkeys daily doses of 10 mg of stilbestrol produced only a slight anemia, although this dosage was four times per kilogram of body weight, the 5 mg dose given to dogs. If the livers of rhesus monkeys were damaged with carbon tetrachloride, stilbestrol produced a decreased hemoglobin percentage and a leucopenia in moderate degree.

Emery, Matthews and Tabrah (1943) showed from experiments on cats and rats that diethylstilbestrol had a slight depressant effect on blood pressure, which was usually of brief duration, and was followed by a return to the previously existing level. The amount of diethylstilbestrol necessary to induce a change in blood pressure was far greater than the dosage needed to induce an estrogenic action. The heart rate was not affected by diethylstilbestrol. They found absorption of the drug from the intestinal tract to be evident in as short a time as three minutes, and to last for about 15 minutes. As far as could be determined, these values were the same for diethylstilbestrol in suspension and in solution.

Edelmann and Gaunt (1942) reported that chorionic gonadotropin did not inhibit lactation in castrate rats. Pregnant mare serum inhibited lactation markedly in intact rats, but not at all

in castrates. An anterior pituitary extract that inhibited lactation in adrenalectomized rats did not do so in normal animals. Estrogens in doses of 1.0 mg per day inhibited lactation in both intact and castrate rats, but much less so in the latter. A dose could be found which would inhibit lactation in intact but not in castrate animals. In a limited series, combinations of estrogen and progestrone or testosterone did not give decisive results. Indirect evidence was given that when the ovary was stimulated by gonadotropin or estrogen, it released nonestrogenic substances which assisted in the inhibition of lactation. They questioned the physiological importance of any direct action of estrogen in inhibiting lactation in the rat.

Lewis and Turner (1942) compared the local application of skin irritants to the local application of diethylstilbestrol as stimulants to mammary growth. They found that unilateral percutaneous treatment of the mammary glands of male rabbits with estrone or diethylstilbestrol caused either greater development of the mammary glands on the treated side, or in some cases, development of treated glands only. Injection of estrone and unilateral percutaneous application of turpentine caused, in two of three rabbits, the development of larger mammary glands on the turpentine treated side. Turpentine treated mammary glands from diethylstilbestrol injected rabbits did not show an increased size over untreated glands. One rabbit, given turpentine alone unilaterally, had several mammary glands showing some duct development on the treated side. This could not be repeated in other rabbits. Other irritants applied to the shaved skin unilaterally gave negative results. The

authors concluded that estrogens possess characteristics and properties not present in skin irritants which superficially appear to cause local subcutaneous hyperemia.

Lewis and Turner (1941-42) compared estrogens and various carcinogenic chemicals as promoters of mammary growth and as lactogens. They found the most active to be stilbestrol, which proved to be at least four hundred times as effective as estrone. Next in activity was estradiol benzoate, which showed 240 times the effectiveness of estrone. Anol and triphenyl ethylene compared favorably with aqueous estradiol, but were only one thirtieth as effective as estrone. Dibenzanthracine was only one seventy-fifth as effective as estrone.

Matthews, Emery and Schwabe (1941) studied the effects of stilbestrol on the reproductive systems of male rats. In some cases the drug was administered with a stomach tube, in others by intramuscular injection. They observed and reported several interesting effects. About two weeks after treatment was begun, the scrotum was noticeably shrunken, and the testes were softer than normal. A few weeks later the testes were located in the abdomen, and the scrotum resembled that of a castrated rat. Interest in female rats in estrus was absent, and copulation was not observed. At autopsy the testes, epididymides, prostates and seminal vesicles appeared markedly atrophied. Histological examination confirmed this observation. Azoospermia and desquamation of the germinal epithelium were observed. Body growth was retarded.

These same investigators (1942) reported on the effects of long continued oral administration of stilbestrol on body growth

and organ weights of adult castrated and uncastrated female rats. They found that body weight increment was seriously impeded. Only slight gains were made by the uncastrated rats, while the castrates actually lost weight. The mean per cent of time in estrus was markedly increased in the case of the normal females, and persistent estrus was established in the castrated rats. The pituitary gland and uterus were significantly increased in weight in both groups; the weights of the thyroid glands, liver, kidneys, and spleen were significantly altered in the castrates, and the ovaries were significantly reduced in weight in the entire females.

Chang (1943) experimenting with stilbestrol on two adult rams reported results somewhat contradictory to those of Matthews and his co-workers on the effects of the drug on the adult male reproductive system. He stated that sperms of two adult rams were

> collected with an artificial vagina twice every day during a period of three months. During this period, stilbestrol was first implanted, then injected and then removed in order to investigate the effects of estrogen on sperm production. Sperms increased seven to nine days after implantation, and the effect lasted five days. The higher the dosage the higher the sperm production. There was no effect on sex drive, sperm morphology, or quality and fertilizing capacity of spermatazoa. Sperms collected from the treated animal showed a higher tendency to agglutinate. It is suggested that the effect on sperm production may be brought about by stimulation of the hypophysis to release secretion.

Folley, Watson and Bottomley (1941) stated that observations on five castrated and three normal immature male goats indicated that teat growth was isometric irrespective of castration. Subcutaneous implantation of, or inunction with diethylstilbestrol or its dipropionate caused an appreciable increase in the rate of

teat growth for a time in both normal and castrated males, but this allometric growth progressively diminished. No externally visible udder growth occurred even when estrogen treatment was continued for over a year, and was supplemented with progesterone or ethinyltestosterone. In young female goats a change from isometric to allometric teat growth often took place at an early age. During the first rutting season, teat growth ceased completely, but allometric growth was resumed when the rutting season ended. Inunction of the udder region with diethylstilbestrol caused an increase in the rate of teat growth accompanied by udder growth in the virgin female, and this was followed by prolonged lactation at the height of which the daily milk yield was 1.5 to 2.0 liters. No treatment with anterior pituitary extract was necessary. During the first part of the artificial lactation period the composition of the milk was that secreted in the colostral period, later the milk was of excellent chemical quality.

During the last three years many reports of the clinical uses of diethylstilbestrol have appeared in the literature. Brownlee (1942) presented a theoretical discussion of the interrelationships between estrogens, progesterone, and the pituitary with their effects on the uterus. He called attention to the fact that when estrogens reach a sufficiently high concentration, they cause regression of the corpus luteum and simultaneously sensitize the uterus to the oxytocic principle of the pituitary body. Should the placenta die, estrogenic level necessary to inaugurate the mechanism of birth might never be reached, and the fetus would be retained. The presence of the fetus in the uterus,

acting as a deciduoma, maintains the corpus luteum, while the corpus luteum maintains the uterine contents. The logical treatment to break this pathological cycle would be to raise the blood estrogen level to that stage at which the corpus luteum regresses. This can be done, according to the author, by injecting 25 mg of stilbestrol.

Frank and Smith (1943) reported the use of 25 mg doses of diethylstilbestrol in two cases of bovine retained placentae. In both cases the placentae were expelled two days later without further treatment. They stated that the results in these two cases warranted further trials of this drug in obstinate cases of retained placentae. They failed to report whether or not they had made a uterine examination to determine the degree of retention before administering the diethylstilbestrol.

The Canadian Journal of Comparative Medicine (1943) reported a case of placental retention following the operative removal of dead twins from a Holstein cow treated with uterine injections of sulfanilamide in mineral oil to guard against sepsis from the decomposing afterbirth. Because of the flaccidity and atonisity of the uterus, 20 mg of diethylstilbestrol were injected intramuscularly on the first and fourth days. On the fifth day it was possible to remove the membranes completely. It was thought that the diethylstilbestrol helped keep the cervix open and furthered the involution of the uterus.

Conn (1944) reported that in his experience the intramuscular injection of 20 mg of diethylstilbestrol was far superior to any other method for the treatment of retained placentae in dairy

cattle. He stated that in 75 per cent of his cases results were secured within 36 hours. Most cases came in heat, he added, within 72 hours. If heat failed to occur within seven days, a second dose was given.

Contradictory observations were reported by Glenney (1944), who found that the drug did not expel bovine placentae, but might be useful supportive therapy in pyometra. He thought that its value to stimulate involution of the uterus following calving was questionable.

Golledge (1942) reported on the use of diethylstilbestrol in a case of hydrops amnii. Results were good, but, since other treatments were simultaneously employed, definite conclusions cannot be drawn.

Gould (1942) in a discussion of the physiological basis for the use of estrogens in veterinary practice, stated that he has found stilbestrol superior to any other preparation in the treatment of anestrus. He used from 10 to 20 mgs intramuscularly. Estrus usually became evident within six days, but conception usually did not occur until the next heat period which usually came on in about three weeks. In five out of six cases checked by rectal examination he found that corpora lutea were present in the ovaries ten days after estrus, but in one case the injection appeared to act as a "trigger mechanism" inducing symptoms of estrus without ovulation. He believed that the animal should be examined to be sure it is not pregnant before stilbestrol is given. He suggested the use of stilbestrol in both non-specific and trichomonad pyometra.

Montgomerie and Brownlee (1941) recommended stilbestrol for anestrus in non-pregnant animals. They suggested dosages of 10 mg to 20 mg for cows, 1 mg for ewes, 5 mg for sows and 2 mg for bitches.

Rowson and Spriggs (1942) reported "great success" in the treatment of pyometra of cattle with stilbestrol in doses of 20-25 mg given intramuscularly. They stated that in suitable cases pus began to be expelled in 24 hours, and continued until the uterus was empty.

Since that time English veterinarians have used stilbestrol and its derivatives quite extensively in the treatment of anestrus, retention of the fetal membranes, mummified fetus, pyometra and retention of the corpus luteum. Few of them have included specific data in their reports, most of which are of isolated clinical cases involving many uncontrolled factors. There is still much work to be done before all of the uses of stilbestrol in cattle practice can be properly evaluated.

Schnelle (1942) reported extensively on the uses of diethylstilbestrol in small animal practice. He has used it successfully in eight of nine cases of urinary incontinence in spayed bitches, in pseudopregnancy, polydipsia, polyuria and enlargement of the prostate. Reports of others have agreed with Schnelle.

Although the subject of bovine retained placentae is one of great economic importance, the basic causes of retention are not well understood, and a review of the voluminous literature finds very little agreement on the therapeutic measures that should be employed in treating the condition.

The general literature on retained placentae is too well known and extensive to be completely reviewed here. Most of it is an expression of the views of the authors with very few reports of critical studies.

Williams, Udall, Frost and their co-workers (1924) made exhaustive laboratory examinations on a group of 75 retained placentae cases, and Udall, Cushing, and Fincher (1925) studied 56 cases. They presented convincing evidence that lesions of the endometrium and the placental surface of the chorion constitute the basis for retained membranes. Many of these lesions, they found, originated at the time the fetal membranes began to form early in embryonic life, and all were present long before the advent of parturition. It was their opinion that the basic causes of retained placenta are intimately associated with errors in animal husbandry, uterine infection and genital ill health. They did not find Brucellosis to be a factor. In one group of 75 animals with retained placentae 55 were negative to the laboratory tests for Brucella abortus. They stated that the view that retained placentae without abortion is evidence of infection with Brucella abortus is not supported by the facts.

Hatch (1941) studied 40 specimens of bovine uteri at various stages of gestation in an attempt to clarify some of the controversial points regarding the anatomy and histology of the uterus during pregnancy, and of the placento-maternal relationships. He concluded that while at all times during gestation there is some intercaruncular epithelium on the uterus that most of it is destroyed during pregnancy. Various stages of epithelial destruction

may be seen at any time during gestation. He found the maternal epithelium of the caruncle to be complete at all times during gestation, and that the placento-maternal relationship in the caruncular areas was entirely epithelichorial.

The caruncle according to Hatch (1941) develops by an overgrowth of the tissues of the lamana propria pushing the epithelium toward the uterine lumen. The connective tissue is invaded by cords of epithelial cells from the surface which form pockets or canals in the caruncle. These pockets are invaded by, and filled with, tissues of the chorionic trophoderm.

Ferguson, Irwin and Beach (1941) studied the blood picture of 24 cows during a 34 day period preceding and a 34 day period following parturition. Twenty of the cows were studied through two normal parturitions, three through one normal parturition and 19 of the same individuals through a Brucella abortus infected pregnancy. They did not state whether or not any of the animals suffered from retained placentae.

They concluded that infection with Brucella abortus does not greatly influence the blood count during the parturient period, since the general trends apparent in both normal and infected cattle were similar. They observed that the number of erythrocytes per cubic millimeter increased slightly immediately following calving, and then decreased somewhat below the pre-calving level in the last 24 days of the post-calving period. The total number of leucocytes decreased significantly immediately following parturition, and later increased to about the pre-calving level.

They found no significant changes in the percentage of either

the neutrophiles or the lymphocytes in the period studied, but did find an increase in the percentage of monocytes and a decrease in the percentage of eosinophiles during the 10 day post-calving period.

Smith and Kilbourne (1893) in their investigations on the nature and etiology of Texas fever of cattle were the first to study the bovine blood picture. They gave the number and size of the red cells and counted the leucocytes, but because of the small number counted did not consider their work accurate.

Dimock and Thompson (1906) studied the blood of normal cows and gave the following values for the total cell count:

	Average number	Minimum number	Maximum number
Erythrocytes per cmm.	6,152,689	4,818,000	7,920,000
Leucocytes	5,486	2,349	10,610

They reported the following values for the various leucocytes elements:

	Average %	Minimum %	Maximum %
Large mononuclears	1.4	0.2	3.3
Polynuclear	30.5	13.0	45.8
Eosinophiles	13.15	3.8	26.5
Lymphocytes	54.2	31.0	76.0
Mast Cells	0.59	0.1	1.2

Burnett (1917) summarizing the work to date gave values agreeing essentially with those of Dimock and Thompson, and

pointed out that there was some apparent fluctuation in values for various parts of the country.

Haden (1940) in his excellent treatise on hematology discussed the clinical interpretation of the blood picture and brought out numerous points pertinent to this discussion. During the short period a granulocyte remains in the blood stream it usually shows no alteration except the changes of degeneration which a few undergo before dying in the circulation. In general, the nucleus, the granules and the cytoplasm remain substantially as they were on emerging from the bone marrow.

The bone marrow, he stated, is affected not only by chemical stimuli from bacterial products in calling out new cells, but these same toxic substances which stimulate new cell formation may also injure the embryonic cells which give rise to new granulocytes. The value of a differential white cell count lies in determining from the number and type of cells present, how great the stimulus for the formation of new cells is, and thus to determine the severity of a toxic process. At the same time it is necessary to evaluate the capacity of the marrow to respond which will depend on the state of the marrow and any toxic effect on it.

The granulocyte count in the blood always reflects a balance between the need of the tissues and the capacity of the marrow to respond. It may reflect the state of the marrow provided the cells are freely released or the need of the tissues if the marrow can freely respond. The bone-marrow varies in state of activity, capacity to respond, and degree of maturation and quality of cells provided by it. The tissues vary in requirements to combat injury,

and the speed with which they take cells out of the blood stream. The blood count shows only a section of a passing parade, the members of which originate in the marrow and constantly leave through the tissues or gastro-intestinal tract, or die by the wayside. The places of the members lost are taken by new ones from the marrow. The number and quality in the parade show the condition of the source of supply, and the need for reinforcements at the point for which they are headed.

If the marrow is affected by toxemia, the cells appearing in the circulation show evidence of such damage. The toxemia may so affect the developing cells in the marrow that normal differentiation does not go on. Thus there may be no nodulation or separation of the nucleus into nodes. Another important evidence of a toxic effect on the developing granulocyte is the appearance of "toxic" or basophilic granules in the cytoplasm. Still other evidences of toxic damage are the appearance of swollen cells, abnormal staining reactions and smudges.

A deficiency in the number of granulocytes in the circulating blood, if not due to aphasia of the marrow or mechanical blocking of the cells in the marrow, is due to depression of myeloid activity by toxins. Granulopenia of this latter type is characterized by the appearance in the circulating blood of granulocytes with immature nuclei and toxic granulation.

EFFECTS OF STILBESTROL ON RETAINED PLACENTAE

All cows used in this experiment were clinical cases presented at the Kansas State College Veterinary Hospital by the

owners for treatment for retained placentae. All breeds common to this area were represented. They included three Holsteins, six Jerseys, one Guernsey, eight Ayrshires, two Shorthorns, 10 Herefords, one Angus, and 12 classified as grades. They varied in age from 19 months to 11 years.

Each case was examined on its arrival by a competent clinician. A careful manual exploration of the uterus was made and if, in the opinion of the clinician, the placenta was so firmly attached as not to come away by itself in a reasonable length of time (48 hours), the case was accepted for the experiment. The condition of the placenta, the degree of retention and the condition of the cervix and vagina were recorded.

Commercial solutions of Stilbestrol in oil were used and the drug was given intramuscularly in all cases. Single doses varied from 30 to 80 mg of pure stilbestrol. In some cases a single dose was given, in others it was repeated from one to three times. In each case a daily manual examination of the uterus was made and the findings recorded. No attempt was made to remove the placenta manually as long as, in the opinion of the clinician, it could be left without endangering the life of the patient. The results of this experiment are recorded in Table 1.

TABLE 1. EFFECT OF STILBESTROL ON 43 CASES OF RETAINED PLACENTA

Case No.	Breed	Age yrs	Estimated wt. in lb.	Hours since calving	Degree of retention	Dose in mg	Times re-peated	Degree of loosening at end of 24 hours	48 hours	72 hours	96 hours	Method of removal	Evaluation of treatment
1	Her.	4	900	48	Firm	40	3	Slight	Slight	Marked		Manual	No benefit
2	Grade	3	800	72	Firm	40	3	None	None	None		Manual	No benefit
3	Grade	7	800	24	Moderate	40	2	Moderate	Extensive	Complete		Unaided	Questionable benefit
4	Hol.	8	1300	60	Firm	80	1	Extensive	Complete			Unaided	Beneficial
5	Grade	3	750	72	Firm	40	2	None	None	Slight		Manual	No benefit
6	Grade	6	850	24	Firm	40	2	Extensive	None	Complete		Manual	Beneficial
7	Jers.	5	700	72	Slight	60	0	Extensive	Complete			Unaided	Beneficial
8	Hol.	10	1700	48	Firm	80	1	None	None	Slight		Manual	Injurious; strained-necrotic vag.
9	Ayr.	5	1100	72	Firm	50	3	None	None	Slight	Moderate	Manual	No benefit
10	Ayr.	4	1050	72	Firm	50	2	None	Slight	Moderate		Manual	No benefit
11	Angus	5	1200	48	Firm	50	2	None	None	Slight		Manual	Injurious; strained for 10 days
12	Her.	4	1000	72	Firm	50	3	None	Slight	Slight	Moderate	Manual	No benefit
13	Her.	7	1100	48	Firm	50	2	None	Slight	Slight	Slight	Manual	No benefit
14	Guern.	6	1200	24	Firm	80	0	None	Slight	Coma		Manual	Injurious; Died in coma
15	Ayr.	5	1100	24	Firm	50	0	None	None	Slight		Manual	Injurious; Necrotic vaginitis
16	S.H.	5	1200	72	Firm	30	2	None	Slight	Moderate		Manual	Questionable benefit
17	Grade	3	800	48	Firm	30	3	None	Slight	Moderate	Extensive	Manual	Questionable benefit
18	Grade	4	850	72	Moderate	30	3	Slight	Moderate	Extensive	Complete	Unaided	Questionable benefit
19	Jers.	4	750	24	Firm	30	3	None	None	Slight		Manual	No benefit
20	S.H.	5	950	72	Moderate	30	2	Slight	Extensive	Complete		Unaided	Questionable benefit
21	Ayr.	2	650	24	Firm	30	3	None	None	Slight	Slight	Manual	No benefit
22	Her.	6	1000	48	Firm	30	0	Extensive	Complete			Unaided	Beneficial
23	Her.	?	1200	48	Firm	50	2	None	Slight	Slight		Manual	No benefit
24	Ayr.	11	1150	72	Firm	50	3	None	None	Slight	Slight	Manual	No benefit
25	Jers.	1½	500	96	Moderate	30	0	None				Manual	No benefit
26	Her.	2	750	48	Firm	30	2	None	None	None		Manual	No benefit
27	Grade	4	750	72	Moderate	50	0	None	Slight			Manual	Questionable benefit
28	Jers.	3	700	72	Firm	30	2	None	None	Slight		Manual	No benefit
29	Grade	7	900	48	Firm	40	3	None	None	None		Manual	No benefit
30	Her.	6	1000	72	Moderate	50	2	Slight	Moderate	Moderate		Manual	Questionable benefit
31	Ayr.	2	750	48	Firm	30	3	None	None	Slight	Slight	Manual	No benefit
32	Grade	9	950	48	Firm	50	0	None	None			Manual	Injurious; Necrotic vaginitis
33	Her.	5	1050	24	Firm	50	3	None	None	Slight	Slight	Manual	No benefit
34	Grade	5	900	72	Moderate	50	2	None	Moderate	Moderate		Manual	No benefit
35	Jers.	9	850	48	Firm	30	3	None	Slight	Slight	Slight	Manual	No benefit
36	Grade	6	1100	48	Firm	40	1	Slight	Moderate	Complete		Unaided	No benefit
37	Ayr.	4	900	24	Firm	40	2	None	None	Slight	Slight	Manual	No benefit
38	Her.	3	1000	72	Moderate	50	2	None	Slight	Slight	Moderate	Manual	No benefit
39	Grade	8	1150	48	Moderate	50	0	Slight	Moderate	Extensive	Complete	Unaided	Beneficial
40	Her.	3	950	72	Firm	50	1	None	None	Slight		Manual	No benefit
41	Hol.	9	1450	48	Firm	50	3	None	None	Slight	Slight	Manual	No benefit
42	Ayr.	5	900	24	Firm	30	2	None	None	Slight		Manual	No benefit
43	Jers.	4	900	72	Moderate	30	1	None	Moderate	Moderate		Manual	Questionable benefit

Results from Use of Stilbestrol on Retained Placentae

Of the 43 cases of bovine retained placentae treated in this experiment four appeared to be definitely benefited by the injection of stilbestrol. Although it is not possible to determine this point with accuracy, it was the opinion of the clinician in charge that the loosening of the placenta was more rapid than is normally encountered in placentae attached with an equal degree of firmness. In eight cases the rate of detachment seemed slightly in excess of that observed in similar untreated cases. These were classified as being questionably benefited by the treatment.

Five cases seemed to be definitely injured by the treatment. Case No. 14, a valuable purebred Guernsey, became comatose 65 hours after the administration of 80 mg of stilbestrol, and passed into a parturient paresis-like coma. She regained consciousness after the administration of 500 cc of 20 per cent calcium boro-gluconate, but relapsed into coma again within an hour. Repeated treatments with calcium boro-gluconate brought similar results. No type of therapy used gave any benefit, and the cow died about 60 hours after the onset of symptoms. Autopsy findings were negative except for some passive congestion of one lung. While it is probable that the connection between the symptoms described and the stilbestrol administration is coincidental, it is also possible that the drug may have produced a profound endocrine upset from which recovery was impossible. No reports of similar cases could be found in the literature.

In four other cases a rather interesting and undesirable

phenomenon occurred. At about the time that symptoms of estrus appeared, in from 24 to 70 hours after treatment began, several of the cows showed some evidence of straining. Examination at this time did not reveal the presence of a vaginitis. Cases 8, 11, 15 and 32 were all mature cows with large well relaxed vulvas which allowed the vagina to evert with each straining attempt. Straining was so violent in each case as to produce defecation. As a result, the vaginal mucosa was contaminated every few minutes with fecal material. Three of these cows developed necrotic vaginitis and the fourth an acute catarrhal vaginitis. All four cases required protracted treatment. Three recovered eventually and the fourth, a valuable purebred Holstein, developed a stricture of the vagina, severe enough to interfere seriously with future breeding. She was subsequently sold for slaughter.

Twenty-five of the 43 retained placenta cases in this experiment appeared to be neither benefited nor injured by the administration of stilbestrol.

Discussion of Results with Stilbestrol

Retained placenta in the cow is a term used somewhat loosely in the literature. All placentae are retained for a short time following parturition. In healthy cows, this period from parturition to expulsion is relatively short in most cases. In the present study cases have been observed in which the entire placenta came away unaided within 30 minutes following parturition. Other cases have been observed in which the expulsion occurred spontaneously, and without treatment, several days after calving. Still

others have been seen in which the placenta remained in the uterus until liquefaction was complete when the semifluid uterine contents escaped slowly over a period of weeks. Between these extremes are found all degrees of retention. Many cases in which manual exploration reveals that the placenta is rather firmly and extensively attached came away unaided a day or two later.

In dairy cows the fetal membranes are so commonly retained from six to 24 hours as to constitute the rule rather than the exception. Examination at this time reveals that in most cases detachment is extensive and progressive, and that slight manipulation causes the whole mass to drop away. This type of case should not be confused with that in which there is extensive and firm retention with no tendency toward detachment after periods ranging from 24 to 72 hours following parturition. In the former there is no marked pathology, and the whole process is so unaccompanied by untoward after effects that it must be regarded as essentially physiological. In the latter type retention is complete and pathological. Williams, Udall, Frost, Cushing, Fincher and Mobie (1924) and Williams (1925) pointed out that this type of retention is due to, and a manifestation of, uterine pathology that existed before parturition. It is this type that makes placental retention a major problem and which requires careful treatment and supervision if the future fertility and even the life of the animal is to be preserved.

Many conflicting reports have appeared in recent years concerning the therapeutic values of stilbestrol as an aid in the expulsion of retained placentae. Those reporting favorably fail to

give the results of a pre-treatment examination, if one were made, or, in most cases, to report the duration of retention before treatment with stilbestrol was begun. Early treatment, without examination, leads to highly optimistic results because it includes so many cases that do not need treatment.

In the cases studied in this report it would appear that stilbestrol had little value as an aid in the expulsion of retained placentae. Of the 43 animals treated only four (9.3 per cent) appeared to be benefited, eight (18.6 per cent) received slight or doubtful benefit from the treatment and five (11.6 per cent) seemed to be definitely injured by the treatment. One of the animals in this latter group died, one became sterile, and three required protracted treatment before recovery ensued.

The straining frequently observed soon after the administration of stilbestrol in these cases is worthy of further study. Since mild irritants are known to produce some aphrodisiac effect, it has often been suggested that estrogens in general produce libido either by sensitizing the vaginal mucosa or by producing a mild irritation. It is possible that the estrogen effect combined with the irritation from the fluid products of placental decomposition resulted in enough stimulus to inaugurate the straining reflex.

Conclusions from Use of Stilbestrol

1. As a result of the above observations it was concluded that stilbestrol, in the manner used in this experiment was of little, if any, value as an aid in the expulsion of retained bovine placentae.

2. Stilbestrol may sometimes be definitely harmful in retained placenta cases.

EFFECTS OF STILBESTROL ON PYOMETRA FOLLOWING RETAINED PLACENTAE

For this experiment 43 cows with retained placentae were selected from herds on which breeding records were kept and on which repeated examinations were made in this study. The placentae were removed manually when examination revealed that it was practical to do so. None was removed in less than 72 hours after parturition, and some remained as long as 148 hours before it was considered advisable to remove them. In no case was the uterus irrigated, and the only other treatment was to place a one ounce gelatin capsule of powdered sulfanilamide deep in each horn of the uterus.

Each case was examined approximately two weeks later. By this time it was found that all symptoms of acute inflammation had subsided, but that in all cases a pyometra was present as evidenced by the presence of pus in the uterus and by retarded involution.

At this time a single dose of from 30 to 50 mg of stilbestrol, depending on the size of the cow, was administered intramuscularly, and the patient was observed daily thereafter until

results were noted. If no results were observed in 72 hours, the treatment was repeated.

All cows in this experiment were bred at the first estrual period after the sixtieth day following parturition and were bred at each succeeding estrus until conception occurred.

For comparison of the results the records of these same herds for previous years were examined and 50 retained placenta cases were selected at random in which the breeding record of the cow was complete following treatment. Data on the post-treatment breeding records of these cows are presented in Table 2.

Table 2. Time from parturition to conception following retained placentae in 50 cows not treated with stilbestrol at any time.

Time from parturition to conception in days	Number of cows conceiving		Cow days
60-70		0	0
70-80		1	75
80-90		2	170
90-100		1	95
100-120		5	550
120-140		8	1040
140-160		14	2100
160-180		6	1020
180-200		5	950
Over 200	7*	1*	228
Total		43	6228
Average days per conception			144.8
Per cent left sterile			14.0

* Seven of the 50 cows were eventually discarded as sterile. The other conceived 228 days following parturition.

Results of Stilbestrol Treatment in Pyometra

Of the 43 cows treated 14 showed signs of estrus behavior in 24 hours after injection, 21 in 48 hours and five in 72 hours, making a total of 40 or 93 per cent that came in heat within 72 hours after the injection of a single dose of from 30 to 50 mg of stilbestrol. Two required a second injection and one aged cow showed only vague symptoms of heat even after a third injection.

All of the 42 cows coming in heat following the first or second injection began expelling pus from the uterus soon after the first symptoms. In from two to seven days from the advent of estrus all uteri were empty of pus and involution proceeded rapidly. Rectal examination during the stilbestrol induced estrus revealed that the tubular genitalia possessed excellent tone and were erectile. Inspection of the cervices through a vaginal speculum revealed them to be open in all cases.

In the one cow in which three injections were needed estrual symptoms were never well pronounced. Several examinations were made during the course of treatment. All showed the cervix to be rather tightly closed and the uterus was atonic at all times. Later a cervical catheter was passed and the uterus irrigated. She finally came into estrus 65 days after parturition. Estrual cycles were fairly regular thereafter but after being bred at each of 10 heat periods, she was finally discarded as incurably sterile.

Forty-two of these cows conceived in from 60 to 140 days following parturition (Table 3). None was bred in less than 60 days from calving. Eight conceived in less than 70 days, 10 in from 70 to 80 days, 11 in from 80 to 90 days, five in from 90 to 100

days, five in from 100 to 120 days, and the remaining three in less than 140 days for a total of 3620 cow days from calving to conception for the 42 cows. The average time required for each conception was 86.2 days.

Table 3. Time from parturition to conception of 43 cows following treatment for pyometra with stilbestrol after retention of the fetal membranes.

Time from Parturition to conception	Number of conceptions	Cow days
60-70 days	8	520
70-80	10	750
80-90	11	935
90-100	5	475
100-120	5	550
120-140	3	390
Over 140	1*	
Total	43	3620
Average days per conception		86.2
Per cent of cows left sterile		2.33

* Sterile

Of the 43 cows one, or 2.33 per cent, failed to conceive and was discarded as incurably sterile. Of the 42 that became pregnant 24 conceived at the first service, 10 required two services and the remaining eight conceived on the third service. The average was 1.62 services per conception.

Of the 50 control cases in Table 2, none conceived in less than 70 days, one in from 70 to 80 days, two in from 80 to 90 days, three in from 90 to 100 days, five in from 100 to 120 days, eight in from 120 to 140 days, 14 in from 140 to 160 days, six in from 160 to 180 days, five in from 180 to 200 days and one at 228 days. Seven, or 14.0 per cent, failed to conceive and were

eventually discarded for that reason. The total cow days for the 43 cows that conceived was 6228. The average time from parturition to conception was 144.8 days as compared to 86.2 days for the stilbestrol treated cows. The difference in the average is 58.6 days.

Discussion of Stilbestrol Treatment in Pyometra

No matter how much care is exercised in removing a retained placenta manually a certain amount of debris is left behind. If bacteria were not already present in the uterus they are introduced on the arm of the clinician. The endometrium is irritated and traumatized by the manipulation and the resulting inflammation gives rise to a catarrhal exudate. The atonic uterus is unable to expel this accumulation which then acts as a deciduoma causing retention of the corpus luteum of pregnancy, closure of the cervix and retention of the putrid, liquefying material. Eventually the corpus luteum regresses, estrus ensues, the uterus is emptied and the condition is relieved. However, the process is a slow one and frequently during the interval the endometrium undergoes so much change as to destroy its ability to support a future conception and the cow becomes sterile. In less severe cases the animal remains in poor health for a long time, breeding is delayed and, in a large percentage of cases, the succeeding pregnancy is pathological.

Several years ago the author attempted treatment of these cases by expressing the corpus luteum. When this was accomplished, estrus followed in a few days and recovery from the pyometra was

prompt. However, it was not always possible to accomplish the enucleation of the corpus luteum, which at this stage has sunk deeply into the ovary, and all too frequently the operation caused enough traumatism to induce an ovaritis. Follow up examinations revealed that in a large percentage of cases the ovary became adhered to the broad ligament, the abdominal wall or the pavilion of the oviduct following this treatment.

The advent of stilbestrol has placed at the disposal of the veterinarian a powerful estrogen that can be marketed at a price that makes its use feasible in those cases where its effects are beneficial. The treatment of the pyometra which invariably follows the manual removal of retained placentae would seem to be one of these.

Treatment of the 43 cows in this experiment with stilbestrol resulted in 42, or 97.8 per cent, of the cows retaining their fertility as compared to 43 out of 50, or 86 per cent, that conceived again in the control group. In the stilbestrol treated cases an average of 86.2 days elapsed between parturition and conception. In the control group the average period from parturition to conception was 144.8 days.

Conclusion from Results of Treatment of Pyometra with Stilbestrol

From the results of this experiment it was concluded that stilbestrol has marked therapeutic value in the treatment of the pyometra which follows the manual removal of retained placentae in bovines.

THE BLOOD PICTURE IN BOVINE RETAINED PLACENTA CASES

One hundred cases of bovine retained placentae, taken as they were presented for treatment, were used in this study. There was no selection of any kind other than that the animal had retained her fetal membranes for at least 24 hours following parturition and that they had not been expelled at the time the blood sample was drawn. Each case was classified according to the duration of retention into 24 hours, 48 hours, 72 hours, etc., up to 192 hours of retention, the longest encountered in this series. Animals were placed in that time grouping which most nearly corresponded to the actual time from parturition to the taking of the blood sample.

In order to determine the normal bovine blood picture for the area of this experiment, blood samples from 15 healthy non-parturent cows were submitted to the same studies, using the same methods and apparatus, as the samples from the retained placenta cases. Each animal in this group was given a careful clinical examination before the sample was drawn. As nearly as could be determined all were in perfect health. All were free from mastitis and negative to the agglutination test for brucellosis.

All blood samples were taken from the jugular vein using California type bleeding needles and B.A.I. type blood tubes, the inside of which had been previously moistened with a saturated aqueous solution of ammonium oxylate.

Hemoglobin determinations were made by diluting 0.5 cc of blood 1 to 20 in a white cell pipette with N/10 hydrochloric acid and reading after 30 minutes in a Haden-Hausser hemoglobinometer.

As soon as each sample was taken, blood films were made on microscope slides and were stained with Wright's stain. Differential leucocyte counts were made on all samples.

Total red cell counts were made at 1-200 dilution with Hayems solution using the improved Neubauer hemacytometer. Total white cell counts were made at 1 to 20 dilution in two per cent acetic acid solution using the same hemacytometer as for the red cell counts. In making the differential leucocyte counts a record was kept of the percentage of neutrophiles showing toxic, or basophilic granulation, and the smudges were classified as none, few, many or abundant.

Results of Blood Counts of Retained Placentae Cases

In the group of normal cows the average red cell count was 5,433,000 per cmm, the lowest was 4,550,000 per cmm, and the highest was 6,700,000 per cmm. The average total white cell count for this group was 6,380 per cmm, the lowest 4,200 per cmm, and the highest 7,800 per cmm.

The average differential leucocyte count for this group as shown in Table 4 was 4.33 per cent eosinophils, 0.6 basophils, 0 per cent myelocytes, 0.2 per cent juveniles, 0.33 per cent stabs, 32.7 per cent segmented neutrophils, 58.3 per cent lymphocytes and 2.9 per cent monocytes. No toxic granulation was observed and smudges varied from none to few.

Twelve cases were encountered in this study in which the time from parturition to the taking of the blood sample placed them in the 24 hour grouping. The average total cell count for this group

TABLE 4. BLOOD PICTURE OF 15 NORMAL COWS

Case No.	Total cell count		Hb.	Differential Leucocyte count									
	R.B.C.	W.B.C.		Eosin.	Bas.	Myel.	Juv	Stab	Seg	T.G.	Lymph	Mono	Smudges
1	5,350	6,400	8.5	4	0	0	0	1	30	0	62	3	None
2	5,950	5,400	9.0	5	0	0	0	0	41	0	53	1	None
3	5,500	9,200	9.0	4	0	0	0	0	34	0	62	0	Few
4	5,475	9,300	10.0	5	2	0	0	1	33	0	55	4	Few
5	5,700	9,200	9.5	2	0	0	0	1	34	0	59	4	None
6	4,750	7,800	9.0	5	1	0	1	0	30	0	59	4	None
7	5,500	6,000	7.75	4	1	0	0	0	24	0	66	5	Few
8	6,700	5,800	9.0	6	0	0	0	0	28	0	65	1	None
9	6,100	6,400	9.5	3	0	0	1	0	31	0	62	4	Few
10	4,950	5,800	8.75	4	0	0	0	1	37	0	53	5	None
11	4,550	4,200	8.0	5	1	0	0	0	34	0	59	1	None
12	5,200	5,000	8.0	4	1	0	0	0	35	0	58	2	None
13	5,050	5,400	7.5	3	1	0	0	1	29	0	62	4	Few
14	5,300	4,800	8.5	6	0	0	1	0	34	0	56	3	None
15	5,475	5,000	9.0	4	2	0	0	0	36	0	44	2	Few
Aver	5,433	6,380	8.73	4.33	.6	0	.2	.33	32.7	0	58.3	2.9	Few

Key:

R.B.C. - Red cell count in thousands
W.B.C. - White cell count in thousands
H.B. - Hemoglobin in gms. per 100 cc.
Eos. - Eosinophils
Bas. - Basophils
Myel. - Myelocytes
Juv. - Juveniles
Seg. - Segmenters
T.G. - Toxic granulation
Lymph. - Lymphocytes
Mono. - Monocytes

was 6,478,000 erythrocytes per cmm and 5,943 leucocytes. The average hemoglobin level was 9.3 gm per 100 cc of blood.

The average differential leucocyte count revealed the leucocytes to be composed of 5.1 per cent eosinophils, 0.75 basophils, 0.15 myelocytes, 0.58 per cent juveniles, 1.83 per cent stabs, 21.18 per cent segmented neutrophils, 63.0 per cent lymphocytes and 4.42 per cent monocytes. Of the neutrophilic leucocytes 4.83 per cent contained toxic granules. Eight of the 12 films showed a few smudges. Complete data on this group are given in Table 5.

There were 14 cows in the 48 hour retention group. As shown in Table 6 the average total red cell count was 5,721,000 per cmm, the total white cell count was 6,280 per cmm and the hemoglobin 8.9 gm per 100 cc. In the differential leucocyte count for this group there was an average of 3.9 per cent eosinophils, 0.85 per cent basophils, 0.43 per cent myelocytes, 1.7 juveniles, 7.1 per cent stabs, 9.2 per cent segmenters, 69.2 per cent lymphocytes and 7.5 per cent monocytes. Toxic granulation was observed in 47.3 per cent of the neutrophils.

Twenty-two cows were in the 72 hours group. The average red cell count was 5,759,000 per cmm, the average white cell count was 5,950 per cmm and the average hemoglobin value was 8.8 gm per 100 cmm of blood. Of the leucocytes there were 6.3 per cent eosinophils, 0.54 per cent basophils, 1.6 per cent myelocytes, 3.9 per cent juveniles, 7.3 per cent stabs, 2.9 per cent segmenters, 71 per cent lymphocytes and 7.1 per cent monocytes (Table 7). Of the various neutrophilic elements 76 per cent showed toxic granulation and smudges were classed from many to abundant in all samples.

In the 96 hour classification there were 16 cases with an average cell count of 5,889,000 per cmm. The average white cell count for this group was 7,250 per cmm and the average hemoglobin was 9.44 gm per 100 cmm of blood (Table 8). The average percentage of various types of leucocytes was 5.4 for eosinophils, 0.69 basophils, 3.6 myelocytes, 9.3 juveniles, 16.6 stabs, 3.25 segmenters, 57.4 lymphocytes and 3.3 monocytes. Toxic granulation appeared in 33.3 per cent of the neutrophils. In six samples smudges were classed as few and in the remaining 10 they were classed as many.

Twelve cows had suffered retention 120 hours when the samples were taken. In this group the averages were 6,002,000 red cells and 6,179 white cells per cmm respectively, and 9.06 gm of hemoglobin per 100 cc of blood. Differential leucocyte averages were 3.83 per cent eosinophils, 0.93 basophils, 3.17 myelocytes, 9.83 juveniles, 15.9 stabs, 6.1 segmenters, 58.0 lymphocytes and 3.5 monocytes. Of the neutrophils 10.7 showed toxic granulation. Eight of the samples showed few smudges, one showed none and the remaining three showed many (Table 9).

In the 144 hour group the average blood picture for the ten cows as shown in Table 10 was 5,643,000 red cells and 6,355 white cells per cubic millimeter, hemoglobin 8.4 gm per cubic centimeter, and the differential leucocyte percentage 2.7 eosinophils, 0.5 basophils, 0.5 myelocytes, 0.8 stabs, 10.5 juveniles, 18.0 segmenters, 53.4 lymphocytes and 3.6 monocytes. Toxic granules appeared in 2.2 per cent of the neutrophils, and smudges were few in four samples and lacking in the other samples.

There were eight cows in the 168 hour group and the average

blood picture was 6,175,000 red cells and 6,000 white cells per cubic millimeter of blood. Hemoglobin was 8.84 gm per 100 cc and the average percentage of the different leucocytes was 3.1 eosinophils, 0.6 basophils, 0.25 myelocytes, 0.12 juveniles, 8 stabs, 30.6 segmenters, 54. lymphocytes and 4.5 monocytes. Toxic granulation was 1.2 per cent and smudges none to few. The data on this group is presented in Table 11.

The averages for the group of normal cows and for the eight groups of retained placenta cases are assembled and can best be studied in Table 13. No significant deviations from the normal were found in the total red cell or white cell counts or in the hemoglobin values for any of the retained placenta groups. Little variation was found in the percentage of eosinophils, basophils, lymphocytes, or monocytes in the retained placenta groups. All values for these elements corresponded closely to those found for the normal cows and to those given by Dimock and Thompson (1906) and Burnette (1917) for normal cows.

In the neutrophilic granulocytes, however, there was a marked deviation from the normal. Values for the segmented neutrophils dropped from an average of 32.7 per cent for the normal to 21.18 per cent for the 24 hour group, 9.2 per cent for the 48 hour group and reached a minimum of 2.5 per cent in the 72 hour group. They rose slightly in the 96 hour group (3.25 per cent) and the 120 hour group (6.1 per cent) and then rapidly, to give values of 18.0 per cent for the 144 hour group and 30.6 per cent and 30.5 per cent for the 168 hour and 192 hour groups, respectively.

The values for the immature types of neutrophilic elements,

the myelocytes, juveniles and stabs, increased from a percentage of 0 for the myelocytes, 0.2 for the juveniles and 0.33 per cent for the stabs in the normal cows to a maximum of 3.17 for the myelocytes and 9.83 per cent for the juveniles in the 120 hour group, and 16.6 per cent for the stabs in the 96 hour group. Their values dropped from this maximum, as that of the segmenters rose, so that in the 192 hour group the entire blood picture closely corresponded to that found for the normal cows.

No toxic granulation was observed in any of the neutrophilic elements in the group of normal cows, but toxic granulation appeared in 4.83 per cent of the neutrophils in the 24 hour group, 47.3 per cent in the 48 hour group, reached a maximum of 76.0 per cent in the 72 hour group, and then dropped steadily to give a value of 1.0 per cent in the 192 hour group. The concentration of smudges corresponded closely to the appearance of toxic granulation, being abundant in the 72 hour group, many in the 48 and 96 hour groups, and few in the others.

When the above described deviations from the normal blood picture were plotted on the same graph (Fig. 1) it was found that the curves of the juveniles and segmenters followed in general the pattern of the toxic granulation curve and that of the segmenter curve was the inverse of the others. This is what Haden quoting Schilling called a "degenerative shift to the left." This degenerative process was reversed and regeneration began between the seventy-second and ninety-sixth hour of retention.

TABLE 5. BLOOD PICTURE IN 24 HOUR PLACENTAL RETENTION

Case No.	Total cell count		Hb.	Differential Leucocyte count									
	R.B.C.	W.B.C.		Eosin.	Bas.	Myel.	Juv.	Stab.	Seg.	%T.G.	Lymph.	Mono	Smudges
1	7,500	6,850	9.5	4	1	0	0	2	26	5.	63	4	Few
2	7,250	8,500	8.5	5	0	0	1	3	24	8.	62	5	Few
3	6,000	7,050	8.5	2	1	1	2	2	23	7.	60	9	None
4	6,575	4,850	9.0	6	0	0	0	3	21	1.	67	3	Few
5	5,550	3,850	9.5	16	1	0	0	1	20	0.	61	1	None
6	6,750	9,550	11.0	4	1	0	1	0	25	10.	69	0	Few
7	6,200	4,850	8.5	4	0	1	1	3	30	0.	57	4	None
8	6,510	6,450	9.5	5	2	0	0	1	27	8.	59	6	None
9	6,200	6,150	8.5	1	1	0	0	4	21	5.	68	5	Few
10	5,975	4,450	12.0	7	1	0	1	2	28	3.	57	4	Few
11	6,625	3,100	8.0	3	0	0	0	1	24	5.	68	4	Few
12	7,600	5,970	9.5	4	1	0	1	0	21	6.	65	8	Few
Aver	6,478	5,943	9.3	5.1	.75	.15	.58	1.83	21.18	4.83	63	4.42	Few

TABLE 6. BLOOD PICTURE IN 48 HOUR PLACENTAL RETENTION

Case No.	Total cell count		Hb.	Differential Leucocyte count									
	R.B.C.	W.B.C.		Eosin	Bas	Myel	Juv	Stab	Seg	%T.G.	Lymph	Mono	Smudges
1	6,750	11,400	8.75	4	1	0	1	7	10	50	70	7	Many
2	5,950	5,950	9.0	5	0	0	2	10	19	80	58	6	Many
3	5,550	5,500	8.5	2	1	1	5	7	6	14	69	9	Few
4	6,200	4,450	8.5	4	1	0	3	11	8	42	66	7	Many
5	4,750	6,500	10.5	9	0	1	7	14	6	43	57	6	Many
6	5,700	4,850	7.5	1	1	0	0	1	11	50	78	8	Many
7	5,200	5,850	11.5	4	2	1	1	5	3	20	78	6	Many
8	6,250	5,750	9.0	2	1	0	0	6	5	54	76	10	Few
9	6,100	4,200	7.75	3	1	0	1	4	9	47	74	8	Many
10	5,500	9,800	9.5	4	1	0	1	7	10	60	70	7	Many
11	6,100	4,850	8.0	5	0	1	1	6	11	54	68	8	Many
12	4,950	6,050	8.0	3	1	0	1	7	12	43	69	7	Many
13	6,050	5,550	8.5	4	2	1	0	8	9	50	73	3	Few
14	5,050	7,250	10.0	5	0	1	1	7	10	55	63	13	Many
Aver	5,721	6,280	8.9	3.9	.85	.43	1.7	7.1	9.2	47.3	69.2	7.5	Many

TABLE 7. BLOOD PICTURE IN 72 HOUR PLACENTAL RETENTION

Case No.	Total Cell count		Hb.	Differential Leucocyte count									
	R.B.C.	W.B.C.		Eosin	Bas	Myel	Juv	Stab	Seg	%T.G.	Lymph	Mono	Smudges
1	4,750	5,500	9.0	7	0	2	4	9	3	80	67	8	Abund.
2	5,475	4,800	9.5	8	1	1	3	6	1	85	67	13	Many
3	5,500	8,200	7.5	7	0	3	5	11	0	50	69	5	Abund.
4	5,700	9,100	8.5	6	0	1	1	7	8	80	67	10	Many
5	6,250	11,000	7.75	10	1	3	6	10	1	75	60	9	Abund.
6	5,500	4,200	10.5	5	0	2	4	9	4	100	70	6	Abund.
7	6,750	5,550	8.0	7	1	2	5	8	3	90	66	8	Abund.
8	5,475	4,850	8.5	4	1	1	4	10	2	94	71	7	Abund.
9	4,950	9,800	9.0	11	0	2	3	6	0	91	70	8	Abund.
10	7,500	4,250	9.5	3	1	3	5	3	1	83	77	7	Abund.
11	6,250	5,200	8.5	5	0	0	6	15	2	76	63	9	Abund.
12	4,850	4,500	8.75	9	0	3	3	7	1	46	69	8	Many
13	5,350	4,450	9.5	7	0	1	2	9	5	68	72	4	Abund.
14	6,500	4,850	8.0	4	1	0	1	2	12	50	74	6	Few
15	7,000	5,200	8.5	10	1	2	7	6	1	61	65	8	Abund.
16	7,075	5,100	12.0	5	0	3	4	7	3	58	72	6	Abund.
17	5,400	3,500	7.5	7	0	2	4	3	0	90	79	5	Many
18	6,750	4,800	11.0	3	1	0	5	2	1	83	81	7	Abund.
19	5,700	5,000	8.5	6	1	1	5	7	2	90	72	6	Abund.
20	5,475	6,100	10.0	5	0	3	4	12	4	82	65	7	Abund.
21	5,500	4,450	7.5	1	1	0	1	5	0	76	89	3	Abund.
22	4,500	9,500	7.0	8	2	1	3	7	2	62	71	5	Abund.
Aver	5,759	5,950	8.8	6.3	.54	1.6	3.9	7.3	2.5	76	71	7.1	Abund.

TABLE 8. BLOOD PICTURE IN 96 HOUR PLACENTAL RETENTION

Case No.	Total cell count		Hb.	Differential Leucocyte count									
	R.B.C.	W.B.C.		Eosin	Bas	Myel	Juv	Stab	Seg	%T.G.	Lymph	Mono	Smudges
1	7,600	6,400	9.5	6	1	4	9	18	4	38	55	3	Few
2	5,500	6,500	12.0	5	0	3	11	15	3	35	58	5	Many
3	7,000	6,850	9.5	7	1	1	4	9	3	70	67	8	Many
4	6,100	5,980	11.0	5	1	2	12	20	2	33	53	5	Many
5	5,350	6,850	9.0	4	0	5	10	16	4	40	57	4	Few
6	5,450	4,850	8.5	8	1	2	9	18	5	25	54	3	Few
7	5,475	5,750	9.5	4	1	6	8	17	4	30	57	3	Many
8	6,700	9,550	9.5	5	0	5	10	19	3	42	56	2	Many
9	6,100	8.750	8.5	4	0	3	8	14	4	38	64	3	Many
10	4,950	5,550	9.0	6	1	5	8	17	4	30	58	1	Few
11	5,300	6,500	8.5	7	2	1	12	20	1	25	53	3	Few
12	6,500	4,450	8.5	5	0	6	11	16	6	20	55	1	Few
13	5,400	6,050	8.0	3	1	3	7	18	3	15	61	4	None
14	5,200	5,500	9.5	5	0	5	12	17	4	33	55	2	Few
15	6,100	7,050	8.75	7	1	3	8	15	3	25	60	3	Few
16	5,500	9,550	10.0	6	1	4	10	17	3	34	56	3	Many
Aver	5,889	7,250	9.44	5.4	.69	3.6	9.3	16.6	3.25	33.3	57.4	3.3	Many

TABLE 9. BLOOD PICTURE IN 120 HOUR PLACENTAL RETENTION

Case	Total cell count		Hb.	Differential Leucocyte count									
No.	R.B.C.	W.B.C.		Eosin	Bas	Myel	Juv	Stab	Seg	%T.G.	Lymph	Mono	Smudges
1	5,475	5,000	7.5	4	1	3	10	16	6	10	56	4	Few
2	5,050	5,400	9.0	5	1	2	9	17	7	17	56	3	Few
3	4,550	9,200	8.5	3	0	3	11	15	8	15	59	3	Many
4	6,100	7,800	8.75	2	0	2	8	14	5	5	67	2	None
5	5,500	4,200	9.5	6	1	4	12	17	6	12	51	3	Few
6	4,750	5,800	10.5	2	2	3	10	15	6	8	61	6	Few
7	7,600	4,750	9.5	4	1	3	9	18	5	18	55	5	Many
8	9,450	6,500	7.5	5	3	4	11	14	8	5	61	4	Few
9	6,750	6,050	8.5	3	0	2	8	17	4	8	65	1	Many
10	5,350	8,750	9.0	4	0	4	10	16	6	10	54	4	Few
11	5,500	5,850	8.5	5	1	3	9	17	4	8	58	3	Few
12	5,950	4,850	12.0	3	1	5	11	15	8	12	53	4	Few
Aver	6,002	6,179	9.06	3.83	.93	3.17	9.83	15.9	6.1	10.7	58.0	3.5	

TABLE 10. BLOOD PICTURE IN 144 HOUR PLACENTAL RETENTION

Case No.	Total cell count		Hb.	Differential Leucocyte count									
	R.B.C.	W.B.C.		Eosin	Bas	Myel	Juv	Stab	Seg	%T.G.	Lymph	Mono	Smudges
1	5,300	7,050	9.0	3	0	1	1	12	20	3	59	4	Few
2	5,200	9,550	12.0	1	1	0	2	8	22	5	63	3	Few
3	7,280	12,000	9.5	5	0	1	0	14	17	0	58	5	None
4	5,590	4,750	10.5	4	0	1	1	13	19	10	60	2	Few
5	6,700	5,550	7.5	2	2	0	0	12	23	0	55	6	None
6	6,100	4,850	8.5	3	0	0	0	10	16	0	67	4	None
7	4,550	8,250	10.5	5	1	2	3	14	21	5	50	4	Few
8	9,250	6,050	9.0	1	1	0	0	6	20	0	69	3	Few
9	6,100	5,500	7.5	3	0	0	1	16	22	0	53	5	None
Aver	5,643	6,355	8.4	2.7	.5	.5	.8	10.5	18	2.2	53.4	3.6	

TABLE 11. BLOOD PICTURE IN 168 HOUR PLACENTAL RETENTION

Case No.	Total cell count		Hb.	Differential Leucocyte count									
	R.B.C.	W.B.C.		Eosin	Bas	Myel	Juv	Stab	Seg	%T.G.	Lymph	Mono	Smudges
1	5,475	6,050	8.0	3	0	0	0	8	30	0	54	5	Few
2	5,350	6,500	8.5	5	0	0	0	7	33	5	51	4	Few
3	6,750	5,950	7.5	2	0	0	0	9	37	2	48	4	None
4	5,300	6,850	11.5	4	1	1	0	5	24	0	60	5	Few
5	5,700	4,850	9.0	3	2	0	1	11	30	3	60	3	Few
6	7,600	5,750	7.75	3	0	0	0	10	25	0	55	7	None
7	5,975	7,050	9.0	2	1	0	0	6	34	0	55	2	None
8	7,250	5,000	9.5	3	1	1	0	8	32	0	49	6	Few
Aver	6,175	6,000	8.84	3.1	.6	.25	.12	8	30.6	1.2	54	4.5	Few

TABLE 12. BLOOD PICTURE IN 192 HOUR PLACENTAL RETENTION

Case No.	Total cell count		Hb.	Differential Leucocyte count									
	R.B.C.	W.B.C.		Eosin	Bas	Myel	Juv	Stab	Seg	%T.G.	Lymph	Mono	Smudges
1	6,280	5,500	8.0	4	0	0	0	6	31	0	56	3	Few
2	6,550	11,250	7.5	3	0	1	0	5	28	0	60	3	Few
3	6,050	4,850	8.5	4	0	0	0	4	34	0	53	5	None
4	8,900	5,550	9.0	5	0	0	0	6	32	6	56	1	None
5	4,850	6,050	8.75	2	0	0	0	5	31	0	58	4	None
6	4,050	4,100	10.0	7	0	0	0	8	27	0	55	3	Few
Aver	6,113	6,217	8.62	4.17	0	.017	0	5.7	30.5	1.0	56.3	3.2	None

TABLE 13. BLOOD PICTURE OF NORMAL COWS AND COWS AT VARIOUS STAGES OF PLACENTAL RETENTION

	Average Total cell count		Hb.	Average Differential Leucocyte count									
	R.B.C.	W.B.C.		Eosin	Bas	Myel	Juv	Stab	Seg	%T.G.	Lymph	Mono	Smudges
Normal	5,433	6,380	8.73	4.33	.6	0	.2	.33	32.7	0	58.3	2.9	Few
24 hour	6,478	5,943	9.30	5.10	.75	.15	.58	1.83	21.18	4.83	63.0	4.42	Few
48 hour	5,721	6,280	8.90	3.9	.85	.43	1.7	7.1	9.2	47.3	69.2	7.5	Many
72 hour	5,759	5,950	8.80	6.3	.54	1.6	3.9	7.3	2.5	76.	71.	7.1	Abund.
96 hour	5,889	7,250	9.44	5.4	.69	3.6	9.3	16.6	3.25	33.3	57.4	3.3	Many
120 hour	6,002	6,179	9.06	3.38	.93	3.17	9.83	15.9	6.1	10.7	58.0	3.5	Few
144 hour	5,643	6,355	8.40	2.7	.5	.5	.8	10.5	18.0	2.2	53.4	3.6	Few
168 hour	6,175	6,000	8.84	3.1	.6	.25	.12	8.	30.6	1.2	54.	4.5	Few
192 hour	6,113	6,217	8.62	4.17	0	.02	0	5.7	30.5	1.0	56.3	3.2	Few

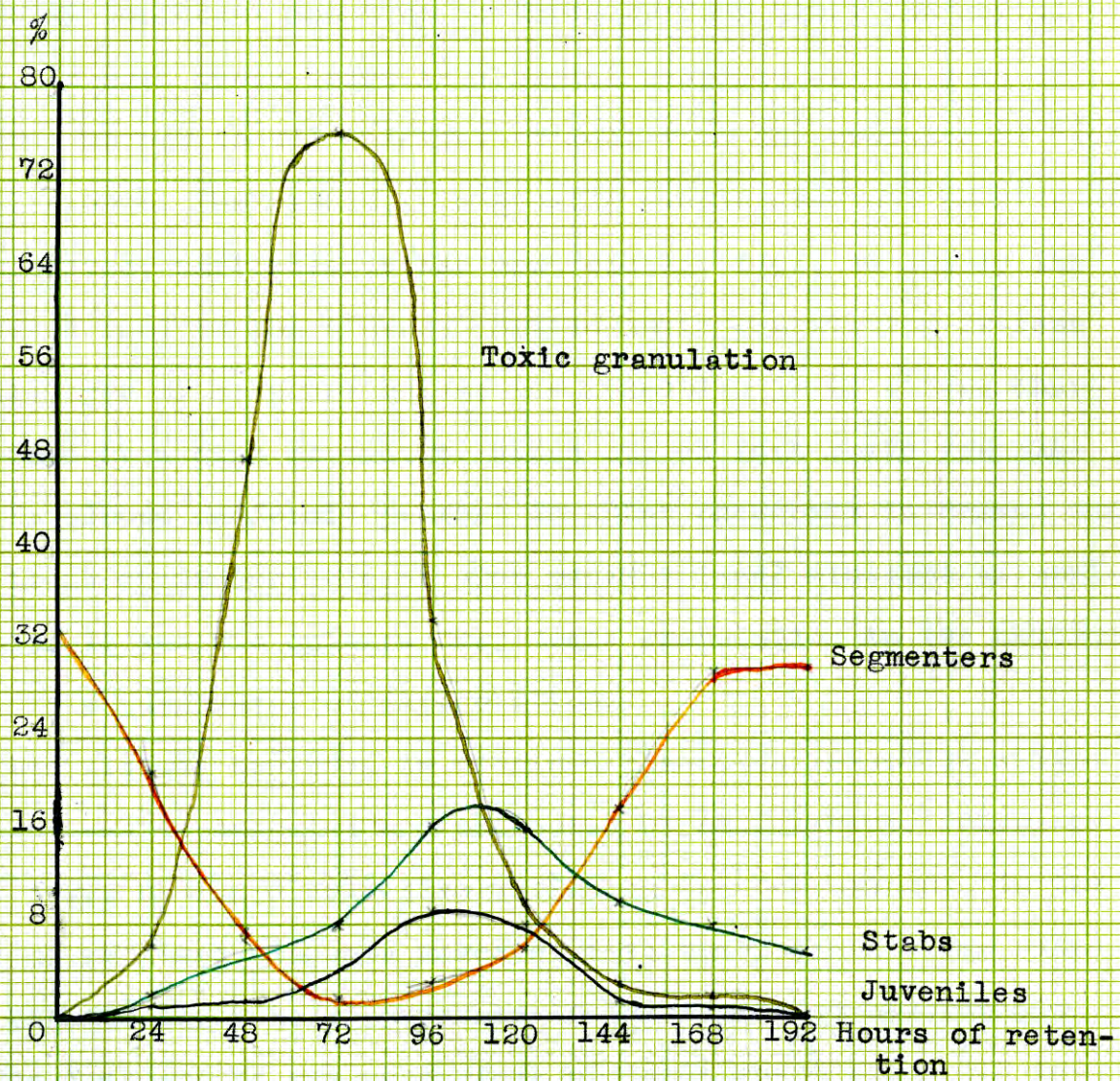

Fig. 1. Percentages of segmenters, stabs, juveniles and toxic granulation at various stages of bovine placental retention, showing a typical Schilling Index degenerative shift to the left. Between the seventy-second and ninety-sixth hour the shift is toward recovery.

Discussion of Blood Picture in Retained Placenta

The blood picture found in bovine retained placenta cases is not at all typical of that found in infective processes in which the response to the infection normally results in an increase in the total leucocyte count up to three or four times the normal value. In such cases there is a normal response on the part of the bone marrow to the chemical stimuli from the products of bacterial growth. The result is a greatly accelerated out pouring of granulocytes, particularly the neutrophilic type, to supply the increased need in the tissues. Little toxic granulation is seen in this type of response unless the infection is of a type characterized by a high mortality. Haden stated that toxic granulation is almost uniformly present in lobar pneumonia in humans and that it is characteristic of such rapidly fatal diseases as peritonitis. It was his opinion that toxic granulation in the neutrophils is a phenomenon of degeneration.

The picture observed in retained placentae resembles closely that seen in toxemias and poisoning by amidopyrine, benzol or other drugs having a selective action on the bone marrow. In the group of retained placenta cases studied there was a close relation between the rate of decrease in the segmented neutrophils and the increase in the appearance of toxic granulation and immature neutrophils. Increases in the numbers of smudges, or cells too abnormal in morphology and staining properties to be identified, were also observed to accompany the decrease in segmented neutrophils and, since the other leucocytic elements showed little if any deviation from the normal values, it is probable

that damaged neutrophils constituted a great proportion of these smudges.

It has been suggested that the decrease in neutrophils in the circulating blood might be due to excessive accumulation of these elements in the uterine walls. However, consideration of the subject would lead one to believe that this is not the case. It is a commonly accepted principle of hematology that the blood count is but a picture of a passing parade and that the number of granulocytes in the blood at any one time reflects the ability of the bone marrow to respond to the needs of the tissues for these elements. Once they have left the circulating blood they do not return to it. The conclusion that the number of granulocytes in the blood at any one time is a good index of their rate of release from the marrow is inevitable in the light of present knowledge.

All of the evidence would seem to indicate that the phenomena observed are the result of a toxemia severe enough to seriously damage the cells in the marrow and to depress the marrow so that the normal number of normal cells are not formed and released.

The placenta, which during pregnancy was an intimate part of the fetus from which it developed and from which it derived its circulation, dies soon after the severing of the umbilical cord. When it is retained following parturition decomposition soon begins and the products of decomposition are held in close contact with the endometrium which at this time is abundantly equipped with an intricate network of capillaries to facilitate absorption

of the waste products of fetal metabolism.

The gradual recovery could be explained by the thickening of the uterine walls, the dimunition of the area of absorption and the decrease in uterine circulation, due to uterine involution, which is rapid after the third day following parturition, even when the placenta is retained. Thus one would expect toxic effects to appear as soon as decomposition of the placenta began and to continue until involution and thickening of the uterus effectively prevented further absorption.

Conclusions from Study of Blood Picture in Retained Placentae Cases

1. Retention of the fetal membranes in the bovine did not cause marked deviations from the normal of the total red cell count, white cell count or hemoglobin levels of the circulating blood.

2. There was no significant effect on the percentages of eosinophilic, basophilic, lymphocytic, or monocytic elements in the circulating blood caused by retained placenta.

3. Retained placenta in the bovine produced marked effects on the neutrophilic elements resulting in a marked decrease in the normal mature segmented neutrophils and a marked increase in the immature myelocytes, juveniles and stabs.

4. This shift toward the immature forms of neutrophils was accompanied by a marked increase in the appearance of toxic granulation in these elements and an increase in the number of smudges.

5. The changes observed were identical with those observed

in severe toxic conditions.

6. Since the symptoms of toxemia, as shown by the blood picture, began soon after the death of the retained placentae, and increased in severity until they reached a peak near the 72 hour period and then subsided gradually as involution of the uterus progressed, the hypothesis that the blood changes observed were due to the absorption of toxins from the decomposing placenta would seem to be justified.

SUMMARY

1. Forty-three bovine retained placenta cases were treated with doses of stilbestrol ranging from 30 to 50 mg. Of these 43 animals, four were benefited, nine were questionably benefited, 25 received no benefit, and five suffered injurious effects. One of the latter died, one was left sterile, and three required protracted treatment before recovery was effected.

2. Forty-three cows were treated with stilbestrol approximately two weeks after the manual removal of retained placentae. Forty-two of these cows conceived following treatment. The average period from parturition to the conception that followed treatment for 42 of the cows was 86.2 days as compared with an average of 144.8 days for 50 controls treated in the past by other methods. One of the treated cows (2.3 per cent) was sterile following treatment. Seven (14 per cent) of the control group were sterile after treatment.

3. Total cell and differential leucocyte counts were made on 100 cases of retained placenta. It was found that there was

a marked decrease in the mature neutrophils, beginning within 24 hours after parturition and reaching its low point at about 72 hours after parturition and then returning to nearly the normal level by the 196th hour following parturition. This decrease was accompanied by an increase in immature neutrophils and the appearance of toxic granulation indicating a degenerative 'shift to the left.' It was suggested that these changes were due to the absorption of toxins from the decomposing placentae.

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