maintained;[3,4] (2) androgens have a reparative influence;[8] (3) the results of one especially favorable case of testis graft in a female showed localized inhibition of the oviduct, more extensive persistence of the wolffian duct.

Some new observations contribute complementary data on the manner of action of the testicular secretion. In 3 males unilaterally castrated between the ages of 19 days 6 hours and 20 days 21 hours, the prostate and the external genitalia are well developed, but the single testis acts less effectively on the primordia of the opposite side. In a fetus with only a right testis, of somewhat under normal size, the vas deferens is present only on the right side, while on the left, a uterine horn persists. It follows that after early unilateral gonadectomy the action of the fetal testis is relatively localized. A single testis or a fragment of a testis (as appears in the case of an incomplete castration) does not seem to undergo compensatory secretory hypertrophy; with regard to this point, it should be remembered that the hypophysis is not indispensable for embryonic sexual differentiation.[9,10,11] Lastly, some of our observations suggest that more testicular secretion is required to completely inhibit the mullerian ducts, than to maintain the wolffian ducts.

Conclusion. In the male rabbit, early fetal castration (before the 21st day) prevents the formation of male secondary sex characters such as the prostate. At a later stage (24 days) established primordia continue their development even after ablation of the testes; the resulting structures are usually subnormal. The effects induced by testicular secretions are sometimes regionally restricted. Earlier observations on the differentiation and persistence of female genital ducts after early orchidectomy are confirmed and extended.

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9 Ancel, P., *Les Hormones Sexuelles*, Colloque Singer Polignac, Paris, 1937, Hermann.

10 Wolff, E., and Stoll, R., *C. R. Soc. Biol.*, 1937, **126**, 1215.

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16072

The Lymphoid Tissue and Antibody Formation.

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Several early investigators in immunology considered the possibility that the spleen was the main site of antibody formation, but conclusive evidence for this was not found. Hektoen,[1] using the well-known fact that X-ray produces a destructive effect on all lymphoid tissue, demonstrated that antibody formation is restrained to a marked degree in X-rayed animals. He concluded from this observation that the spleen, lymphoid tissue and bone marrow were the principal sites of immune bodies. Murphy and Sturm[2] confirmed Hektoen's findings but considered that they had eliminated the bone marrow as of first importance in the process. This conclusion was based on the fact that depletion of the lymphoid tissue was effected by repeated, small doses of X-ray of low penetration which produced no detectable damage to the bone marrow or other tissues. Yet animals treated in this manner were found deficient in the production of antibodies. In addition, these investigators demonstrated that rabbits, after stimulation of the lymphoid system by dry heat treatment, had an enhanced ability to produce immune substances. Hussey[3] in this

1 Hektoen, L., *J. Infect. Dis.*, 1915, **17**, 415; 1918, **22**, 28; 1920, **27**, 23.

2 Murphy, J. B., and Sturm, E., *J. Exp. Med.*, 1925, **41**, 245.

3 Hussey, R. G., PROC. SOC. EXP. BIOL. AND MED., 1921, **19**, 22.

laboratory found that if the lymphoid system of guinea pigs is kept in a depleted state by X-ray following a sensitizing injection of horse serum, the animals show no anaphylactic manifestations on receiving a second injection of serum. In such animals the antigen continues free in the circulation as long as the X-ray is continued. McMaster and Hudack[4] have clearly demonstrated that antibodies may be formed in lymph nodes but their experiments did not eliminate the possibility that cells other than lymphocytes participated in the reaction. Ehrich and Harris[5] have extended these observations and on the basis that the tissue response accompanying the formation of antibodies in the nodes is predominately a lymphoid one, they consider these cells as the important factor in the development of immune substances. Harris, Grimm, Martens and Ehrich,[6] in a study of the antibodies in the lymph, found when the lymphocytes are separated from the lymph plasma that the titer of the cell extract was substantially and consistently higher than that of the lymph plasma. This is taken to indicate that the lymphocytes either produce antibodies or take them up from lymph plasma. As no evidence for the latter was found, they believe that the lymphoid cells are the responsible agents. Dougherty, Chase and White[7] noted that the antibody content of the blood is increased following the administration of adrenal cortical extract, a fact which they attributed to the destructive action of the hormones on the lymphocyte with the release of the antibodies supposedly formed in the cytoplasm of these cells. Philips, Hopkins and Freeman[8] found no such increase when the lymphoid tissue is destroyed by nitrogen mustard and such treatment may result in an actual depression of the production of antibodies.

In the present investigation an estimate has been made of the antibody formation in animals with extreme hypertrophy of the lymphoid system which follows the removal of the adrenal glands. The release of the antibodies from the lymphocyte under these conditions would be due to the normal shedding of the cytoplasm as the cell matures. In the absence of the adrenals there can be no question of the cortical hormones playing any part in the reaction.

Circulating Lymphocytes in Adrenalectomized Rabbits. The circulating lymphocytes show a definite but not marked rise following removal of the adrenals. This increase is in the average range of 4000 cells per mm^3, which is considerably less than that observed for mice and rats. However, if adrenalectomized rabbits are given an antigen, the lymphocyte count shows a marked increase up to an average of over 14,000 cells while normal rabbits under the same condition have only a slight increase.

Antibody Formation in Adrenalectomized Rabbits. Experiment 1. The adrenals were removed from 2 rabbits and immediately afterwards these animals and normal controls were given 10 cc of horse serum intraperitoneally. Ten days later, all were bled and the sera tested for precipitins. The sera from the adrenalectomized rabbits gave massive precipitates in the low dilutions, and definite positive reactions in dilutions of 1 to 640 in one serum and in dilutions of 1 to 2560 in the other. The serum from the control gave far less precipitate in low dilutions and there were no precipitates in dilutions higher than 1 to 320.

Exp. 2. The adrenals were removed from 7 rabbits and between 2 and 3 weeks later these with four controls were given five intravenous injections of horse serum, consisting of 1 cc, at 2-day intervals. All of the rabbits were bled 10 days following the last injection and the sera tested for precipitins. The results are given in Table I. It will be noted that all of the sera from adrenalectomized rabbits gave definite precipitates in dilutions up to 1:2560 and 4 were positive in 1:5120. Only

4 McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1935, **61**, 783.

5 Ehrich, W. E., and Harris, T. N., *J. Exp. Med.*, 1942, **76**, 335; *Science*, 1945, **101**, 28.

6 Harris, T. N., Grimm, E., Mertens, E., and Ehrich, W. E., *J. Exp. Med.*, 1945, **81**, 73.

7 Dougherty, T. F., Chase, J. H., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 28; 1945, **58**, 135.

8 Philips, F. S., Hopkins, F. H., and Freeman, M. L. H., *J. Immunol.*, 1947, **55**, 289.

TABLE I.

Immunized with 5 Injections of 1 cc Horse Serum Intravenously at 2-Day Intervals, 2-3 Weeks After Adrenalectomy

Dilutions	1:1	1:25	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
1	+		+	+	+	+	+	+	+	+	+	+	+
2	+		+	+	+	+	+	+	+	+	+	+	+
3	+		+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+

Intact Rabbits Given 5 Injections of 1 cc Horse Serum Intravenously at 2-Day Intervals.

Dilutions	1:1	1:25	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
8	+	+	+	+	+	+	+	+	+	+	+	+	−
9	+		+	+	+	+	+	+	+	+	−	−	−
10	+	+	+	+	+	+	+	+	−	−	−	−	−
11	+	+	+	+	+	+	+	−	−	−	−	−	−
12	+	+	+	+	+	+	+	+	−	−	−	−	−

one control serum was positive in dilution of 1:2560 and in the others there was no reaction above 1:320. Far more striking than the difference between the two groups shown by dilution, is the difference in the volume of the precipitates in the lower dilution (Fig. 1).

Exp. 3. This experiment was carried out to compare the development of agglutinating antibodies in adrenalectomized and intact rabbits. A 24-hour slant culture of *Salmonella enteritidis* (mouse typhoid) was washed off with saline and the suspension heated on 3 consecutive days for 15 minutes, at 56°C. In preliminary tests it was found that while intact rabbits would withstand the intravenous inoculation of 1/10 of a culture, the adrenalectomized ones were killed by 1/1000 given intravenously and by 1/100 of a culture inoculated subcutaneously. The following method of immunization proved successful. Two adrenalectomized rabbits and two controls were each given 5 subcutaneous inoculations at 4-day intervals, starting with 1/300 of a killed culture and gradually increasing the amount up to 1/10 for the last inoculation. The animals were bled 10 days after the last injection and the sera tested for agglutinins against the organisms. The sera from the intact rabbits agglutinated up to dilutions of 1 to 40 and 1 to 80, respectively. The serum from the first adrenalectomized rabbit reacted at dilutions of 1 to 640 and the other was definite at 1 to 1280, the highest dilution used.

Effect of Adrenal Cortical Hormones on Antibody Formation in Adrenalectomized Rabbits. The marked over-development of the lymphoid system which follows removal of the adrenals may be prevented by the administration of adrenal cortical hormones. In the following experiment, the effect of suppressing the lymphoid development by the hormone treatment was tested on the antibody formation.

Two adrenalectomized rabbits were given 23 daily injections of 1 cc each of adrenal cortex hormones in oil, which was equivalent to 40 rat units or 2 mg of 14-oxy-11-dehydrocorticosterone (Upjohn Co.). This covered the period following the operation and extended through the period of immunization

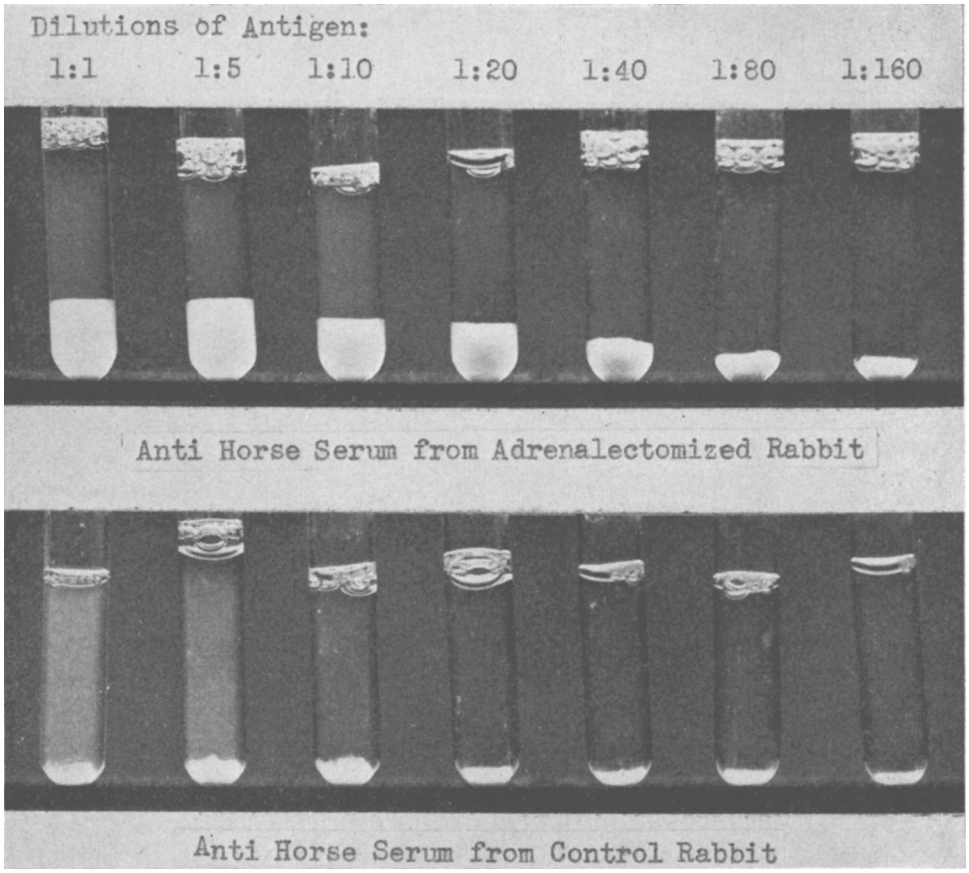

FIG. 1.

with horse serum. A third rabbit was given daily doses of one cc of Eschatin (Parke Davis and Co.). Each cc of this preparation is equivalent to 50 dog units. These animals with 2 untreated adrenalectomized rabbits and 2 intact controls were immunized with horse serum as in the previous experiment. The test of the sera showed no material difference in precipitin formation in hormone treated and untreated adrenalectomized rabbits. The intact controls gave far less response as in the preceding test.

Discussion. Ehrich and Harris[5,6] in a series of papers have adequately discussed the evidence that the lymphocyte is a factor in antibody formation. The experiments reported above are essentially a confirmation of our earlier observation,[2] that animals with hypertrophied lymphoid systems have an enhanced ability to produce antibodies. In the earlier tests dry heat was used as the stimulus; in the present study advantage was taken of the well-known fact that extensive overdevelopment of the entire lymphoid tissue follows removal of the adrenals. If the immune substances are present in the cytoplasm of the lymphocyte and there is evidence for this belief, the release into the circulation is probably due to shedding, which is known to occur as the lymphocyte matures. One of our tests confirms the observation of Dougherty, Chase and White[7] that the release of antibodies may be definitely augmented by adrenal cortical hormones. The present experiments demonstrate that the hormone is not essential for this release and therefore, strictly speaking, antibody production is at least not entirely under the control of the adrenals.

Summary. Adrenalectomized rabbits with hypertrophy of the lymphoid tissues produce

antibodies in amounts far in excess of that produced by intact animals. This is evident not only by definite reactions in higher dilution but by the more massive precipitate in low dilutions. The prevention of the lymphoid hypertrophy by administration of adrenal cortical hormones does not reduce the amount of antibodies in the sera. This is probably due to the disruptive action of the hormone on the lymphocyte with a more rapid release of the immune globulin than would normally take place.

16073

Prevention of Experimental Arteritis in Dogs by Vitamin E.*

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In a recent publication[1] data were presented to show that the combination of a specified high fat diet and experimentally induced renal insufficiency in dogs is regularly followed by predictable lesions in the arterial system. The lesions have been found in elastic arteries, in muscular arteries, and in arterioles in practically every organ and tissue of the body with the exception of the kidney and liver. Essentially the lesion is an acute necrotizing arteritis whose closest human counterparts are periarteritis nodosa and rheumatic arteritis.

When it was found that the dietary factor was lipid in nature, the question naturally arose as to the effect of vitamin E—the so-called "fat anti-oxidant" vitamin—on these experimental arterial lesions.

Methods. The methods have been detailed in previous publications.[1] Briefly these consist of feeding selected table scrap to which is added each day 3.0 cc/kg of a commercial grade of cod liver oil. After this diet has been consumed for 8 weeks or longer, the kidneys are severely damaged by any one of several methods (heavy metal injury, bilateral nephrectomy, *Leptospira Canicola* — usually uranium nitrate, 10.0 mg/kg injected subcutaneously) and the arterial system is examined both grossly and histologically when the dogs die or are sacrificed days to weeks later.

In the first series of 4 dogs vitamin E—one capsule containing 20 mg of mixed natural tocopherols—was given by mouth between 8 and 10 o'clock in the morning and the dogs were fed the diet of table scrap and cod liver oil around 4 o'clock in the afternoon. Following kidney damage the dogs usually quit eating on the fourth to seventh day. Administration of vitamin E capsules was continued until the dog died.

In the second series of 4 dogs vitamin E (in the same form as above) was not started until *after* the kidneys were damaged. The dogs were fed table scrap and cod liver oil for 8 weeks or longer, then a lethal dose of uranium nitrate was injected and a single capsule of 20 mg mixed natural tocopherols per day by mouth was started 0, 24, 48, and 72 hours after the heavy metal injury. Vitamin E therapy was continued until the dogs died in uremia.

In these 8 dogs the single capsule of 20 mg of mixed natural tocopherols amounted to 2.5 to 4.0 mg/kg/day.

Experimental Observations. The significant data on the 4 dogs fed vitamin E concomitantly with the specified high fat diet for 8 weeks or longer before kidney damage was

* This work was aided by a grant from The John and Mary R. Markle Foundation. The mixed natural tocopherols and helpful suggestions were furnished by Distillation Products, Inc.

1 Holman, R. L., and Swanton, M. C., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 87.