The effect of the corticosteroid compounds on the delayed hypersensitive state of tuberculosis has been well documented. *In vivo* studies(16-18) have demonstrated the ability of these compounds to alter the tuberculin reaction and Vollmer(20) noted reversal of tuberculin skin tests following treatment with steroids. Leahy and Morgan(21) reported suppression of the *in vitro* cellular reactivity to tuberculoprotein, indicating that the compounds have a direct action at the cellular level.

The observations presented here provide further evidence of the similarity of the delayed hypersensitive state in tuberculosis and mumps by demonstrating a protective action of corticosteroids on cytotoxicity of mumps viral antigen for hypersensitive cells *in vitro*.

*Summary.* Experimental mumps virus infections in guinea pigs are characterized *in vivo* by a positive delayed hypersensitive skin test reaction to viral antigen and *in vitro* by a cytotoxic effect of mumps virus antigen on cells from tissues cultivated from mumps-infected animals. Cortisone protects against this *in vitro* cytotoxicity of mumps virus.

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## Increased Follicle-Stimulating Activity in the Plasma of Ovariectomized Rats.* (24125)

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Several investigators, using the technic of parabiosis, have been able to demonstrate that bilateral castration results in increased gonadotrophic activity in blood plasma of male and female rats(1). Emery(2) was able to show this increased activity by injection of castrate male serum into immature rats, and also reported on the basis of a few preliminary tests that serum from castrate, multiparous female rats showed gonadotrophic activity. Donor animals used were rats sacrificed over a 2 year period for various other purposes, and no attempt was made to standardize the post-operative period. The present study, in which immature hypophysectomized female rats

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have been used as test animals, reports the presence of follicle-stimulating activity in plasma as early as 7 days after castration and a gradual increase as the post-operative interval is extended to 4 months.

*Methods.* Virgin rats of the Long-Evans strain, averaging 233 g in body weight and 101 days of age, were bilaterally ovariectomized and maintained on stock diet XIV† for varying post-operative periods, *i.e.*, 1 and 2 weeks, 1, 2 and 4 months prior to autopsy. Normal animals of comparable age and body weight were used as onset controls. Blood was taken in heparinized syringe from abdominal aorta while animals were under light ether anesthesia, and was centrifuged under light mineral oil for 30 minutes at 3000 rpm. Minimal amounts of heparin had to be used as a slight excess resulted in subcutaneous hemorrhages and often death of test animal. The pooled plasma for each post-operative period was kept under oil at 4°C for 6 hours or less, so that each test animal was able to receive 4 equally divided doses at 2 hour intervals daily regardless of total amount of plasma injected. Fresh plasma was taken daily, although preliminary studies indicated that there was little if any loss of follicle-stimulating activity when plasma was kept at 4°C for several days. Castrate donors were examined macroscopically at time of autopsy to check completeness of ovariectomy. Plasma from castrated and normal rats was assayed for follicle-stimulating (FSH), and interstitial-cell stimulating (ICSH) activity in immature female rats hypophysectomized at 26 to 28 days of age and 12 to 20 days post-operative. Total doses of 4 cc, 8 cc, 16 cc, 24 cc, and 32 cc of donor plasma were given and the critical doses repeated at least once. Plasma was injected subcutaneously 4 times daily at 2 hour intervals as divided injections were necessary to insure survival when higher dosage levels were given. A 96 hour test period was used and autopsy performed 24 hours after last injection. Uteri and ovaries (minus oviducts were weighed and the ovaries fixed in Bouin's fluid for histological study. Completeness of hypophysectomy was checked by examination of the sella turcica under binocular microscope at time of autopsy. Histological responses for FSH activity and ICSH activity were determined by use of criteria described by Evans *et al.*(3). The minimal effective dose (MED) for follicle stimulation was that amount of plasma required to cause beginning antrum formation and increase in size of follicles above hypophysectomized control levels in two-thirds of test animals. These follicles are described as small-medium (sm) in the Tables. The MED for interstitial cell stimulation was that amount which caused partial repair of "deficient" interstitial tissue in two-thirds of the rats, *i.e.*, resumption of normal nuclear pattern in these cells with some increase in cytoplasm.

*Results.* The data presented in Table I show that FSH activity was detected in plasma of all rats after castration but not in plasma of normal onset controls at highest dosage level used. Within one week after removal of ovaries, injections of 32 cc (total dose) resulted in stimulation of follicular development in somewhat less than two-thirds of the test animals. Since the response was small follicles in one-half the test ovaries and small-medium in the remainder, this was considered to be slightly less than the minimal effective dose. A total dose of 32 cc of plasma from rats castrated for 2 weeks resulted in stimulation of follicular growth (small-medium follicles) in two-thirds of test animals, which by definition can be considered the minimal effective dose. When animals had been castrated for approximately one month, injection of 24 cc of plasma resulted in small follicles in some test ovaries and small-medium in others, a response less than the minimal effective dose, whereas a higher dosage (32 cc) showed a much greater response (small-medium to medium follicles) or more than the minimal effective dose. Therefore, the MED was considered to fall between 24 and 32 cc for this post-operative period. Similarly, with 2 months post-operative period, the MED was considered to fall between 16 and 24 cc, and a

† Stock diet XIV is composed of 68.5% ground whole wheat, 10% fish meal, 10% alfalfa leaf meal, 5% casein, 5% fish oil (Sardiline), and 1.5% iodized NaCl. Lettuce given *ad libitum* twice weekly.

TABLE I. Follicle Stimulating Activity in Blood Plasma of Normal and Castrated Female Rats.

| Post-operative period, days | Total plasma dose, cc | No. test animals* | Uterus wt, mg† | Ovaries‡ | | FSH activity | FSH activity, MED/100 cc plasma |
|---|---|---|---|---|---|---|---|
| | | | | Wt, mg | Follicles | | |
| 0 | 0 | 14 | 17 | 7 | s | Neg. | |
| | 8 | 9 | 16 | 7 | s | " | |
| | 16 | 9 | 18 | 7 | s | " | |
| | 24 | 6 | 17 | 9 | s | " | |
| | 32 | 7 | 18 | 10 | s | " | |
| 7 | 0 | 12 | 17 | 8 | s | Neg. | <3 |
| | 24 | 8 | 16 | 8 | s | " | |
| | 32 | 12 | 19 | 9 | s-sm | <MED | |
| 14–17 | 0 | 9 | 16 | 7 | s | Neg. | 3 |
| | 24 | 6 | 19 | 7 | s | " | |
| | 32 | 6 | 17 | 8 | sm | MED | |
| 31–38 | 0 | 13 | 15 | 7 | s | Neg. | 3–4 |
| | 24 | 11 | 19 | 8 | s-sm | <MED | |
| | 32 | 11 | 25 | 11 | sm, few m | >MED | |
| 60–65 | 0 | 10 | 14 | 7 | s | Neg. | 4–6 |
| | 8 | 6 | 16 | 6 | s | " | |
| | 16 | 8 | 16 | 7 | s | " | |
| | 24 | 8 | 37 | 14 | m, few ml | >MED | |
| | 32 | 6 | 48 | 14 | m-ml | " | |
| 117–128 | 0 | 9 | 16 | 8 | s | Neg. | 6–12 |
| | 4 | 8 | 17 | 8 | s | " | |
| | 8 | 7 | 19 | 8 | s | " | |
| | 16 | 8 | 18 | 11 | sm, few m | >MED | |
| | 24 | 7 | 40 | 15 | m, few ml | " | |
| | 32 | 6 | 46 | 14 | sm-m, few ml | " | |

* Immature female rats, hypophysectomized 26-28 days, 12-20 days post-operative.

† Uterus was dissected free of oviducts and drained of fluid before weighing.

‡ s = small, m = medium, l = large—refer to size of follicles measured with calibrated eyepiece micrometer. Standards are those used by Wooten *et al.*(4). s = up to 375 $\mu$, sm = 450-500 $\mu$, m = 550-650 $\mu$, ml = 700-750 $\mu$, l = 800-1000 $\mu$.

MED—minimal effective dose.

4 month post-operative period showed a further increase as the MED dropped to a point somewhere between 8 and 16 cc of plasma. The gradual increase in FSH activity with increased post-operative period can easily be seen when approximate FSH activity per 100 cc of plasma is calculated (Table I).

As judged by morphology of interstitial tissue in hypophysectomized recipients, no evidence for ICSH activity was found in plasma of either intact or castrate animals. However, increased uterine weights indicating production of estrin (which many investigators consider requires the presence of both ICSH and FSH), were observed when plasma from castrate animals 1 to 4 months post-operative was given.

The results confirm the findings of Emery (2) and the brief mention by Goto(5) and Martins(6) that increased gonadotrophic activity in blood of castrate rats can be demonstrated by direct injection of plasma into test animals. The findings are also in agreement with those obtained through the technic of parabiosis, *e.g.*, Biddulph *et al.*(7), and show that increased gonadotrophic activity in castrate female rats is predominantly follicle-stimulating. The latter investigators found evidence for increased FSH activity in the circulation as early as 3 days post-operative. In addition, our data demonstrate a gradual but progressive increase in circulating FSH activity with increasing post-operative periods up to 4 months.

It should be mentioned that growth promoting activity, as measured by the standard tibia test,‡ was present when 24 and 32 cc levels of both normal and castrate rat plasma

‡ Right tibias of hypophysectomized test animals

were tested in immature hypophysectomized recipients. The growth-stimulating potency of normal rat plasma was equivalent to that recently reported by Contopoulos *et al.*(9).

*Summary.* Follicle-stimulating activity was detected in blood plasma of adult female castrate rats as early as 7 days post-operative by injection of plasma into immature hypophysectomized female rats for a 4-day period. Follicle-stimulating activity continued to increase slowly but progressively as the post-operative interval increased to a 4 months' period. With this bioassay method no direct evidence for circulating interstitial cell-stimulating activity was found at the highest dosage level tested.

were split mid-sagitally, fixed in formol, and stained with $AgNO_3$(8). The uncalcified portion of proximal epiphyseal cartilage was measured with micrometer eyepiece and average widths of 200 $\mu$ or greater (i.e., approximately 50% increase over uninjected control widths) were considered evidence for growth-stimulating activity.

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## Relationship of Fluoride and Dietary Fats to Serum Cholesterol in Rats.* (24126)

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It was shown(1) that doses of 0.5, 1 or 2 mg fluoride as aqueous solutions of sodium fluoride daily did not alter normal serum cholesterol level in the rat. Since Miller and Phillips(2) have found an enhancement of fluoride toxicity in rats on a diet containing 15% cottonseed oil, further work pertaining both to fluoride toxicity and serum cholesterol in which different sources of dietary fat are used seemed indicated.

*Methods.* A total of 150 weanling male Sprague-Dawley strain rats were divided equally into 5 series. Series A was divided into 3 groups, all receiving a stock sucrose diet. Group I animals received no fluoride in their drinking water, Group II received fluoride as sodium fluoride at concentration of 30 $\mu$g F/ml in the drinking water, and Group III received 2 mg of fluoride daily (as sodium fluoride) by stomach tube. Each of the 4 remaining series was divided similarly into 3 groups and received the same amount of fluoride as Series A animals either in drinking water or by stomach tube, but the type of dietary fat was changed. The animals in Series B received cottonseed oil, those in Series C corn oil, Series D Crisco, and Series E lard. Fat was added at a level of 20%. Animals in Group I and III of each series received a fluoride low (F = 0.05 $\mu$g/ml) drinking water *ad libitum*. Composition of the experimental diets which were also available *ad libitum* are summarized in Table I. All animals were housed individually in raised screen cages in air-conditioned room and were weighed twice each week throughout experiment of 10 weeks. Serum cholesterol was determined at beginning of experiment and at weekly intervals (except for 6 and 9 week period) by methods previously described(1).

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