body-temperature were comparable to those in the other experiments. Ox bile injected intraperitoneally is extremely irritating. Rat 27 was in a moribund condition when the last body-temperature was taken and died shortly thereafter. Blood lactic-acid values for all the rats receiving ox bile rose sharply and continued to rise for the duration of the experiment.

Discussion. The body-temperature and blood lactic-acid curves following the intraperitoneal injection of India ink (Fig. 1), of pentothal sodium (Fgi. 2) and of India ink plus pentothal sodium (Fig. 3) are much alike. There is no evidence that the treatment caused increases in blood lactic acid. The initial high values for lactic acid found in most of the rats were surprising, particularly in view of the fact that the excitement, which was the probable cause of these high values, was not otherwise apparent in the behavior of the animals. The second value taken 25 minutes later was in some cases less than half of the initial value and must be taken as the "normal" value for the individual rat. There is little evidence from the lactic-acid curves that the subsequent hourly handling and bleeding produced comparable excitement. All the rats had been handled frequently and were gentle. This experience emphasizes the care with which "initial" values in experiments on rats must be judged.

Following the intraperitoneal injection of 0.5 cc of ox bile plus pentothal sodium, the response is different. There is a greater variation in the degree of hypothermia, a variation that seemed to reflect the physical condition of the animal. Rat 26 died at the end of 2 hours. In all instances there was a pronounced increase in the blood lactic acid.

We believe that these experiments give further support to our belief that the fall in body-temperature following the intraperitoneal injection of particulate substances is not a reflection of shock.

Summary. Adult white rats were treated in 4 ways. In 3 of these, (a) intraperitoneal injection of 2 cc of India ink, (b) subcutaneous injection of 0.2 cc pentothal sodium per 100 g, and (c) treatment (b) followed in 20 minutes by treatment (a), blood lactic acid remained normal but body-temperature fell. In the fourth treatment, subcutaneous injection of pentothal sodium followed in 20 minutes by intraperitoneal injection of 0.5 cc ox bile, a similar fall in body-temperature occurred which was accompanied by a marked rise in blood lactic acid. The rise in blood lactic acid is taken as an indication of shock.

These experiments indicate that the hypothermia produced by the intraperitoneal injection of India ink is not secondary to shock.

Received August 3, 1950. P.S.E.B.M., 1950, v75.

Observation of a Spreading Factor Present in Mammary Gland Extracts.*† (18206)

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It is well known that at the onset of pregnancy a phase of rapid growth of the lobule-alveolar system of the mammary gland is initiated. At this time there is not only an extension of the major ducts but also a rapid proliferation of side buds which invades the adjacent subcutaneous fatty connective tissue (fatty pad). In order to facilitate the forward growth of the end buds, it seemed possible that a mucolytic enzyme or collagenase might be elaborated by the growing mammary gland cells which, through liquefication

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station. Journal Series No. 1223.

† The hyaluronidate and hyaluronidase used in this investigation were generously provided by the Schering Corporation, Bloomfield, N. J.

of the adjacent connective tissue, would make possible the penetration of these growing buds. Several spreading factors, including hyaluronidase, have been found to act in a manner similar to the one described(1-4).

The present investigation was initiated to explore the possibility of finding such an enzyme or factor.

Methods and materials. Both male and female adult albino rats weighing from 150 to 250 g were used. In removing the mammary glands, the animals were skinned, leaving the glands attached to the skin. The blood was squeezed out, then the fatty pads containing the mammary glands were carefully dissected excluding all lymph nodes and other tissue. The stage of pregnancy of each female was determined by examining the size of the fetuses. Since at times it was impossible to extract the glands immediately, some were held at −15°C until this could be done. Twenty-five ml of 0.1 M sodium acetate buffer pH 6.0 with 0.15 M sodium chloride was added to the glands and the material homogenized in a Waring blendor. The homogenate was then centrifuged for 20 minutes at 2,000 r.p.m. The milky supernatant fluid was then pipetted off, avoiding as far as possible the inclusion of any fraction of the fat layer which formed on top.

A test for spreading factors was first tried. This involved the injection of 0.2 ml of the test material and 0.1 ml of 2% T-1824 (Evans Blue) intradermally into a shaved white rabbit. The dye served as an indication of the extent of fluid spread. This test is only a slight modification of several suggested for the assay of hyaluronidase(5,6) The test material in most cases was the mammary gland extract diluted 10×; however, testicular extract diluted 10× and the acetate sodium chloride pH 6 buffer were used as controls. The injections produced blebs on the rabbits' skin which were measured immediately after injection and which were uniformly circular. After 30 minutes the length and width of the blebs were measured again. The net area was then determined.

A turbidimetric method of assay was also employed(7). Several preliminary assays conducted with purified hyaluronidase indicated the reliability of potency of the enzyme preparation and the reproducibility of the method. Before the hyaluronidase assays of the mammary gland preparations could be made, they had to be extracted with either acetone, alcohol or ether to remove the lipids and to precipitate the biologically active material. The precipitate was suspended in 25 ml of the acetate sodium chloride pH 6 buffer, and after stirring 10 minutes the suspension was filtered through a No. 1 filter paper. These filtrates were then assayed.

Experimental. Initial spreading type tests on mammary gland extracts from 5 castrate female rats and 5 females in normal estrous cycles failed to give any indication of the presence of a spreading factor; however, the mammary gland extracts from females pregnant 6 to 11 days produced definite spreading on the rabbits' skin. Several testicular extracts used as the positive controls gave comparable spreading effects, while the acetate sodium chloride pH 6 buffer injections which did not spread were the negative controls. As the initial tests on the extract of mammary glands from pregnant females indicated a spreading factor, it was decided to try the turbidimetric method of assay for hyaluronidase in order to determine if the spreading effect was due to this enzyme. Eight turbidimetric assays on a sample of purified hyaluronidase which gave positive results served as a check. Ten mammary gland extracts from pregnant female rats which gave a spreading effect on rabbits' skin‡ were turbidimetrically assayed. In no

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TABLE I. Spreading Factor Present in Rat Mammary Gland Extracts.

Type of extract	Spreading type assay			
	No. of animals	Initial bleb, mm	Final bleb, mm	Area of spread, sq mm
pH 6 buffer	14	14×14	15×15	22.8
Testis extr.	17	14×14	24×26	336.0
Castrate ♂	5	14×14	15×15	22.8
" ♀	5	13×14	14×14	11.0
Normal ♂	5	13×13	14×14	21.2
" ♀	5	13×14	15×15	33.8
1 day pregnant	3	14×14	15×15	22.8
2 days pregnant	2	13×13	14×15	32.2
3 " "	4	14×14	16×18	72.3
4 " "	3	14×14	18×18	100.6
5 " "	4	13×13	19×20	166.6
6 " "	4	14×14	20×20	160.3
7 " "	4	14×14	20×20	160.3
8 " "	3	14×14	20×21	176.0
9 " "	3	13×14	20×21	187.0
10 " "	5	14×14	21×21	199.7
11 " "	3	13×13	21×22	231.0
12 " "	4	14×14	23×24	279.7
13 " "	3	14×14	22×23	243.6
14 " "	3	14×14	22×22	226.3
15 " "	3	13×14	20×21	187.0
16 " "	4	14×14	20×20	160.3
17 " "	2	14×14	19×19	128.9
18 " "	3	14×14	16×18	72.3
19 " "	3	13×13	14×15	32.2
20 " "	2	14×14	15×15	22.8
21 " "	2	13×14	14×15	22.0

case did these mammary gland extracts give any indication of hydrolyzing the substrate hyaluronidate (Table I).

Since the results of the assays on the mammary gland extracts indicated that the spreading factor was not hyaluronidase, all further assays employed the spreading factor technic. Mammary gland extracts from 5 normal male rats and 5 castrate males gave no indication of a spreading factor.

Assays of mammary gland extracts from 67 female rats taken during various stages of pregnancy indicated the presence of measurable amounts of spreading factor except during the first and last 2 days of pregnancy. The amounts of spreading factor extracted from the mammary gland increased gradually with each day of pregnancy until the maximum was reached on about the twelfth day following which there was a gradual decline in the level of the spreading factor during the remainder of pregnancy.

It was next deemed necessary that the enzymatic character of the spreading factor be checked. Tests using the spreading method of assay were made to determine the inactivation temperature of the mammary gland spreading factor. Testicular spreading factors were run simultaneously.

Mammary gland extracts from 5 pregnant females and 5 testicular extracts were heated to 55°C in a water bath for 10 minutes. None of the extracts were inactivated by these conditions. Five different mammary extracts from pregnant rats and 5 more testicular extracts were heated to 60°C in a water bath for 10 minutes. The testicular extracts gave no spreading effect while the mammary extracts were still active. Tests of a number of mammary gland and testicular extracts which had been heated to 70°C and higher showed complete inactivation.

Discussion. In view of the experimental data it seems rather certain that increased amounts of a spreading factor are present in mammary gland extracts from pregnant female rats, while the glands of normal male and female rats, as well as castrated animals, contained little of this factor. The failure of the spreading factor to hydrolyze hyaluronidate by the turbidimetric method of assay and the difference in the inactivation temperatures of the mammary gland spreading factor as contrasted to the testicular spreading factor indicates rather definitely that the mammary gland spreading factor is not hyaluronidase.

The assay of mammary gland extracts of pregnant rats indicated that the highest level of spreading factor elaboration occurred at the time lobule-alveolar growth is known to be at a maximum.

It appears probable that a mucolytic enzyme or collagenase is elaborated by the cells of the growing mammary gland. Several mucolytic enzymes have been shown to have no hydrolytic action on hyaluronidate(2-4). Further, tumors which have long been reported to contain a spreading factor(8) after

‡ Data on the spreading effect included in Table I.

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much controversy have been reported not to contain hyaluronidase(9). Experiments are now in progress to determine the role of estrogen and progesterone upon the elaboration of the spreading factor by the mammary gland.

Summary. 1. Spreading type assays made on extracts of mammary glands taken from female albino rats pregnant from 3 to 18 days indicated the presence of a spreading factor. The concentration of the factor increased until about the twelfth day followed by a gradual decline. 2. Extracts of mammary glands from normal male and female rats, as well as castrate animals, assayed by the spreading method failed to exhibit any spreading effect at the dilution employed. 3. Turbidimetric assays of mammary gland extract showed that the spreading factor did not hydrolyze the substrate, hyaluronidate. 4. Tests showed that after heating at 60°C for 10 minutes, the spreading factor was still active but inactive after heating at 70°C for 10 minutes.

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Received August 7, 1950. P.S.E.B.M., 1950, v75.

Action of Adrenocorticotropic Hormone (ACTH) in Experimental Allergic Encephalomyelitis of the Guinea Pig. (18207)

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Recent observations in the experimental (1,2) and clinical(3) fields suggest that administration of ACTH inhibits allergic reactions. The type of encephalomyelitis elicited in the guinea pig by the injection of brain emulsion with adjuvants(4-7) provides a convenient laboratory example of an allergic condition since it can be produced easily with a single inoculation of antigen, follows a regular pattern, and shows distinctive clinical and pathological features. Therefore, it was considered of interest to investigate the effect of ACTH upon the occurrence and severity of this type of experimental allergic encephalomyelitis.

Material and methods. Eight groups of guinea pigs, each weighing 300-350 g were given a single injection of rabbit-brain emulsion with adjuvants in the manner previously described(5). Two groups served as controls and received no further treatment, whereas the other 6 groups received injections of ACTH. A purified hormone preparation was used which was assayed for ACTH potency by the ascorbic acid depletion method of Sayers(8). A typical assay of potency on this

TABLE I. Assay of Potency of ACTH Preparation

Dose inj. per 100 g rat body wt, γ	Decrease in ascorbic acid mg/100 g adrenal tissue
0.5	43
2.0	112
8.0	158

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