effects the phospholipids in these tissues.

Summary. 1. The lipid phosphorylation affects of a single dose of choline were compared in rats maintained on low protein-high fat diet or similar diet supplemented with methyl acceptor, guanidoacetic acid. 2. Administration of a single dose of choline stimulated lipid phosphorylation in the kidney, heart and aorta.

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Release of a Histamine-Destroying Factor During Anaphylactic Shock in Guinea Pigs.* (26658)

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Rose and Leger(1) reported that a substance capable of destroying histamine was released in rabbits during anaphylactic shock. Code and associates(2) demonstrated such a substance, probably histaminase, in the blood of white rats during anaphylactic shock. The present study was done to determine whether a similar histamine-destroying factor is released during anaphylaxis in guinea pigs.

Methods and materials. A group of 22 guinea pigs (700 to 1100 g) of both sexes of our mongrel laboratory strain was sensitized by 4 subcutaneous injections of crystalline egg albumin given at intervals of 24 to 48 hours. The animals were used 3 weeks to 3 months after these sensitizing injections.

During the studies of anaphylaxis, the animals were anesthetized by intraperitoneal administration of pentobarbital sodium in a dose of 3 mg per 100 g of body weight. A catheter of No. 60 polyvinyl tubing was inserted into the right atrium *via* the right jugular vein for withdrawal of samples of blood. After a control sample had been taken, 2 mg of crystalline egg albumin in 0.3 ml of an isotonic solution of sodium chloride was given *via* the catheter. Three to 5 minutes later, a specimen of blood was drawn while the animal was in shock. All samples of blood were drawn in a syringe that had

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been rinsed with a solution containing 1000 U.S.P. units of heparin per milliliter. A portion or all of each sample of blood, whether obtained as a control or after shock, was extracted immediately for histamine(3) and subsequently assayed on an isolated segment of atropinized guinea-pig ileum. The flask containing one of the control samples broke during the extraction process.

Six of the 21 control specimens were divided into 2 parts; one part was immediately extracted for histamine, and the other was incubated for 1½ hours in a constant-temperature water bath at 37°C and then extracted. Seven of the 22 shock specimens were divided into 2 parts and treated similarly.

Four additional shock specimens were divided into 3 parts; one part was extracted immediately, and to another was added 0.05 ml of Tyrode's solution per milliliter of blood, after which it was incubated as above and extracted; to the third part was added 0.05 ml of aminoguanidine solution (10 mg/ml) per milliliter of blood, after which it was incubated and extracted as for the second part.

All of the final extracts were assayed on an isolated segment of atropinized guinea-pig ileum. The incubation time of 1½ hours was chosen initially for these experiments because, in studies on the rat, Code and associates(2) had found that most of the destruction of histamine was evident in that period. To check on the necessity for this incubation period, after the above studies were completed, blood specimens from 6 of the second group of 11 guinea pigs in shock were divided into 4 parts; the first part was extracted immediately, whereas the second, third and fourth parts were incubated at 37°C for ½, 1 and 1½ hours, respectively, then extracted and assayed.

Blood from 11 of the animals was drawn at varying intervals after the intra-atrial injection of the antigen, namely 15 to 30 seconds, 1 to 1½ minutes, 2 to 2½ minutes, and 4 minutes. The blood for each withdrawal period was divided into 2 parts; one was extracted immediately and the other after incubation for 1½ hours at 37°C.

TABLE I. Blood Histamine* of Sensitized Guinea Pigs before and after Shock.

Animals	Before shock	After shock: Immediate extraction	After shock: Incubation† followed by extraction	Loss of histamine after incubation, %
1	.06	.30	.085	71
2	.06	.32	.04	87
3	.07	.07	.05	0
4	.025	.34	.04	88
5	.075	.21	.057	72
6	.05	.425	.105	75
7	.06	.11	.03	73
8	.08	.16	.08	50
9	—	.57	.09	84
10	.13	.91	.135	85
11	.06	.66	.05	92

* Expressed as μg of histamine/ml of whole blood.

† Incubated for 1½ hr at 37°C.

Results. Anaphylactic shock developed in all the animals. The first indication of such shock was seen 20 to 30 seconds after intra-atrial injection of the antigen, when the animal's lips and nose began to twitch vigorously. At about 1 minute, a sudden and violent jerking forward of the head occurred. The heart rate rapidly increased. Breathing became labored and inspiration was extremely difficult. Artificial respiration was sometimes necessary. Dyspnea increased during the second minute. Tachycardia persisted. Several of the animals displayed increased peristalsis, and some lost urinary and fecal control.

With only one exception (animal 3), all shock specimens showed a 2-fold to 14-fold increase in concentration of histamine over the control specimen (Table I). All shock specimens except in animal 3 showed a loss of 50 to 92% of the histamine after 1½ hours of incubation.

The destruction of histamine was as great after ½ hour of incubation as after 1½ hours. Concentration of histamine in the control samples of blood was the same whether they were extracted immediately or after 1½ hours of incubation.

The 4 specimens of blood from animals in shock that were incubated with aminoguanidine showed no loss of histamine.

As noted previously, 22 specimens from 11 of the animals were secured at various in-

tervals after the shocking dose was administered. Twelve of these showed an increase in histamine as compared to values in the same animal before shock; 19 specimens showed evidence of a histamine-destroying factor. Among those specimens taken 15 to 30 seconds after injection of the antigen, a 3-fold increase in histamine was noted in one animal, a 50% increase in another and no increase in 3 animals; destruction of histamine was demonstrated in 4 of these 5 specimens. For the specimens obtained 1 to 1½ minutes after injection of the antigen, only one of the 3 animals showed an increase of histamine, and 2 of the 3 showed evidence of destruction of histamine. For those specimens taken 2 to 2½ minutes after injection of the antigen, 4 of 6 guinea pigs showed a 2-fold to 14-fold increase in histamine; each of the 6 specimens showed evidence of destruction of histamine. Four minutes after injection of the antigen, 6 of 8 animals showed a 50% to 7-fold increase in histamine; 7 of the 8 showed evidence of destruction of histamine.

No specific index of degree of clinical shock was used. However, it was evident that some animals displayed a more severe degree of shock than did others. Neither the height of blood histamine present 4 minutes after injection of the antigen nor the degree of increase of this value over control values could be correlated with the estimated degree of shock observed in the animals.

Comment and conclusions. A state of shock can be induced by intravenous administration of the specific antigen into guinea pigs sensitized to crystalline egg albumin and anesthetized with pentobarbital sodium. The finding that histamine is released into the blood of guinea pigs during anaphylactic shock(4) has been confirmed.

It has been shown previously that aminoguanidine blocks the action of histaminase (5,6). Since aminoguanidine blocked the destruction of histamine in this study, it has been concluded that the factor in the blood responsible for destruction was diamine oxidase (histaminase). Code's group(2) arrived at a similar conclusion from their observations on rats.

The incubation period necessary to demonstrate destruction of histamine in blood from shocked guinea pigs is much less than that in rats. A period of 1½ hours in all specimens was used initially in this study because of prior experience with rats. However, shorter incubation was tried after the first part of the study was completed. Destruction was as extensive at ½ hour as at 1½ hours. The work of Arunlakshana and co-workers(7) suggests that the rate of destruction is even more rapid than this.

No histamine-destroying activity was demonstrated in blood secured before shock. However, such activity was present in shock blood regardless of the extent to which the histamine was increased. Blood drawn at varying intervals after the shocking dose showed histamine-destroying activity even when little or no increase in histamine could be demonstrated. This was especially true of the specimens secured at 15 to 30 seconds.

However, Boreus(8) found that blood histamine increased within 30 seconds after injection of the antigen. He also noted a reduction of about 50 per cent in the tissue mast cells at this time. The prompt appearance of histaminase activity at a time of massive destruction of mast cells suggests that this activity may have been released by these cells. This possibility merits further study.

The inconsistent increase of blood histamine in the first 2 minutes after injection of antigen could be caused by concomitant release of histaminase. This also may account in part for the failure to find a correlation between increase in blood histamine and estimated degree of shock in the animals.

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Influence of Graded Levels of Progesterone in Ovariectomized Rats upon Placentomata Formation Measured by Total DNA.* (26659)

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Weichert(1) was first to produce placentomata (deciduomata) reaction in ovariectomized rats. Astwood(2) and Rothchild and Meyer(3) suggested its use in assay of progesterone, whereas Evans *et al.*(4) proposed use of this reaction in assay of luteotropic hormone. Use of DNA in quantitative study of mammary gland growth(5) suggested that a quantitative assay of progesterone or luteotropin might be developed under standardized conditions comparing DNA of normal and traumatized horns of rat uteri. It was thought that studies of the various hormones which might influence growth of the placentomata should be conducted in normal animals before hypophysectomized would be used. Present study concerns influence of graded amounts of progesterone (P) on placentomata formation.

Materials and methods. Sprague-Dawley-Rolfsmeyer rats fed Purina Lab Chow were maintained in animal room at 78 ± 1°F until regular estrous cycles were established. Ovariectomy (OE) was performed when rats showed fully cornified smears. Immediately after operation, progesterone was given at level of 1.5 mg/day from day 1-5 during "pretrauma period"(3). Endometrium of one uterine horn of each animal was traumatized on day 6 by inserting needle through cut tubal end of uterus as far as cervix on this side. By withdrawing needle slantwise, antimesometrial aspect of endometrium was scratched 2 times throughout its entire length by sharp needle point. Immediately after operation, P was given daily at levels of 1, 2, 3, and 6 mg respectively from day 6-9, "post-trauma period"(3), whereas control rats did not receive P during this period. Rats were killed on day 10, 24 hours after last injection. Pituitaries, adrenals and uteri were removed rapidly, transferred to ice-cold vials, weighed on Roller Smith balance and put in deep freezer. Uteri were divided before weighing into control and traumatized horn and processed for DNA(6). Traumatized horns were cut in pieces, washed in alcohol before starting alcohol and ether extraction in order to obtain dry-fat-free tissue (DFFT).

Results. P in graded doses stimulated a graded increase in mean total DNA in the traumatized uterine horns. One mg P increased total DNA of traumatized horn markedly as compared to rats which did not receive P during post-trauma period. Six mg P did not further increase total DNA of traumatized horn as compared to 3 mg group (Table I). Decrease of DNA/mg DFFT took place in all groups. Nine of 23 rats receiving no P during post-trauma period had placentoma reactions better than group average of 7% increase of traumatized horn over control horn. A few rats did not show reactions under influence of P. Per cent increase of wet weight, DFFT and DNA of trauma-

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