taining 0.005 mg of gramicidin per cubic centimeter of medium.

Suspensions of 1% washed sheep's cells containing 0.01 mg of gramicidin per cubic centimeter were completely hemolyzed after one hour in a water bath at 37.5°C. Hemolysis was complete in 24 hours in tubes containing 0.001 mg of gramicidin per cubic centimeter of red cell suspension. The addition of small amounts of pooled guinea pig serum caused a slight but definite diminution of the hemolytic activity of gramicidin. The destruction of complementary activity of the serum by heating to 56°C for 30 minutes did not alter the inhibition of hemolysis. Attempts to neutralize this hemolytic effect by the addition of varying amounts of cholesterol to solutions of alcohol and suspensions of gramicidin were unsuccessful.

Further studies must be made to determine whether or not there is a relationship between the hemolytic effect of gramicidin and the hemolysin produced by the soil bacillus from which gramicidin is extracted. The description by MacLeod, Mirick and Curnen[4] of the effect of the intravenous administration of gramicidin on animals makes it seem likely that the hemolytic activity of gramicidin may play an important part in the toxicity of this substance. How far the hemolytic effect accounts for the total toxicity must be determined by *in vivo* experiments; however, the hemolytic activity of gramicidin is great enough in the presence of constituents of the blood to render inadvisable the intravenous use of this substance in bactericidal concentrations.

Summary. Gramicidin has a powerful hemolytic action against rabbit's and sheep's erythrocytes *in vitro*. This activity is marked even in the presence of serum, plasma and tissue extract. The presence or absence of complement did not appear to affect the process.

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In vitro Maintenance of Mammalian Endocrine Organs.

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The cultivation of endocrine organs was undertaken primarily with the hope that the secretory activity of such organs and their

[4] MacLeod, C. M., Mirick, G. S., and Curnen, E. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 461.

response to hormones might eventually be studied *in vitro,* under conditions probably less complicated than those occurring within the organism. In view of the contradictory nature of the results reported in the literature, it was first necessary to find a suitable reproducible method by which organs could be maintained structurally for varying periods of time. Recently, Parker[1] has described such a technic which has yielded good results for small fragments of such organs as liver, spleen, lymph node and thyroid. This method, which involves a fluid medium and the daily introduction of mixtures of oxygen and carbon dioxide, appeared best suited for our purpose. Results of the application of this method to the cultivation of thyroid, testis, ovary, certain reproductive accessories, anterior lobe of the pituitary and adrenal from immature and adult rats, guinea pigs and rabbits are given in Table I. Each experiment generally lasted 4 or 5 days. The flasks containing the tissues were kept at 38°. Microorganism contamination cases, which were seldom encountered in the entire investigation are not taken into account. All failures were due to unknown causes.

The results given in Table I indicate that the methods described by Parker for the maintenance of adult tissues in a fluid medium may be successfully applied to certain of the endocrine organs. It also becomes apparent that different endocrine organs exhibit dif-

TABLE I.

Organ	Total No. exper.	No. successful*	Comments on successful experiments
Thyroid	20	16	Both follicular and interfollicular tissue in excellent condition.
Testis	15	11	Maintenance only; no advancement in spermatogenesis or development of interstitial tissue.
Ovary	15	7	Mature follicles, eggs and corpora lutea maintained 10 days.
Epididymis	8	7	Epithelium and connective tissue normal.
Fallopian tube	9	9	
Anterior hypophysis	20	17	Both chromophilic and chromophobic elements well maintained. Areas of growth occasionally noted.
Adrenal cortex and medulla	11	0	
Adrenal capsule	4	0	

*In each experiment, for all organs studied, the glands of a different animal were employed. By a successful experiment is meant one in which all or the greater number of cultured pieces were found in good condition.

[1] Parker, R. C., *Methods of Tissue Culture,* N. Y., Hoeber, 1938.

ferent degrees of sensitivity to alterations in the environment. For example, we have found that thyroid structure can be maintained under a variety of conditions, *i. e.,* in plasma clots, in fluid media containing a small amount of serum or composed entirely of serum and at temperatures ranging from 25-38°C. The adrenal, on the other hand, cannot be maintained under *in vitro* conditions which are favorable for thyroid and pituitary, and apparently requires the presence of additional unknown materials. Mitotic figures and areas of proliferation were encountered only in the case of the anterior lobe of the pituitary. It would thus appear that the medium employed by Parker and in the present experiments is not only satisfactory for the maintenance of such organs as the thyroid, testis, and reproductive accessories, but also permits further growth of the anterior lobe of the pituitary.

In agreement with the results obtained by Parker, the use of autologous serum in our experiments was of no special advantage. In certain cases, sera obtained from other animals of the same species proved to be as satisfactory as serum from the same animal. In other experiments, degeneration occurred despite the use of autologous serum. It was also found that rabbit and guinea pig organs were more successfully cultured than those of rats and that, in most cases, young animal tissues thrived to a better extent than those from adults.

A puzzling observation was the frequent occurrence of many well maintained organ pieces lying near fragments showing almost complete necrosis despite the fact that all were of about the same size and continually bathed by the same medium. Perhaps different degrees of injury sustained by the organs in the cutting process may account for this finding.

The failure of adrenal capsule tissue to proliferate to any extent *in vitro* was surprising in view of the marked regeneratory capacity of this tissue within the organism. The rather inconsistent results obtained with the ovary are also difficult to understand. It is quite possible that the addition of pituitary organ pieces or extracts to the culture medium might enable such endocrine glands to be more successfully maintained. Our most immediate problems are thus concerned with determining the environmental conditions which are optimal for the culturing of the different endocrine organs.

Summary. 1. The methods of Parker which involve the use of a fluid medium and a high oxygen tension have been applied to the *in vitro* maintenance of endocrine organs. Best results were obtained with fragments of thyroid, testis, epididymis, Fallopian tube and the

anterior lobe of the pituitary of immature and adult guinea pigs and rabbits. 2. Inconsistent results were obtained with ovary. Adrenal tissue and capsule invariably underwent degeneration. 3. Evidences of growth (areas of proliferation and mitotic figures) were observed only in the case of the pituitary. 4. It is concluded that the conditions under which one endocrine organ may be successfully maintained *in vitro* are not necessarily the same as those required by another.

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Failure of Histaminase to Prevent Anaphylactic or Histamine Shock in Guinea Pigs.

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The increasing interest in the hypothesis that anaphylactic shock and allergy depend on the liberation of histamine has directed attention to the possibility of destroying this physiologically active substance *in vivo*. Best and McHenry[1] in discussing the enzyme, histaminase, suggest as its possible rôle the inactivation of histamine *in vivo*. In a more recent note, these authors[2] object to the misinterpretation of this suggestion and deny that available preparations of histaminase are, in their experience, effective *in vivo*.

Karady and Browne[3] were able to protect guinea pigs from the intraperitoneal injection of histamine by earlier intraperitoneal injection of histaminase. They were also able to protect sensitized guinea pigs from anaphylactic shock by treating the animals with histaminase prior to giving the shocking dose of antigen. These injections were given intraperitoneally.

The purpose of this report is to present experiments dealing with the *in vivo* action of a commercial preparation of histaminase* on anaphylactic and histamine shock in guinea pigs.

Methods. Guinea pigs averaging 350 g were sensitized by in-

1 Best, C. H., and McHenry, E. W., *J. Physiol.*, 1930, **70**, 349.

2 Best, C. H., and McHenry, E. W., *J. A. M. A.*, 1940, **115**, 235.

3 Karady, S., and Browne, J. S. L., *J. Imm.*, 1939, **37**, 463.

* The histaminase used was a preparation of the Winthrop Chemical Company (Torantil-T360), obtained through the courtesy of the Department of Medical Research of that company.