Registration. (1) The height of the float indicating rate of flow may be visually noted from time to time. (2) Its position may be recorded on kymograph or photographic paper by means of a sighting attachment which is manually made to follow the movements of the top edge of the float.

Typical Records. Reproductions of original records are presented, showing the augmentation of blood flow in the left circumflex artery during temporary asphyxia (artificial respiration removed), Fig. 4, and in the femoral vein during an inspiratory attempt (trachea closed) Fig. 5.

Summary and Conclusions. The rotameter has been used to measure cardiac input and mean blood flow in the arteries and veins of the anesthetized dog. Typical records are shown. Tests indicate that in routine use the instrument will give reliable blood flow values with an error of less than 10%. Its use enables the experimenter to determine at a glance the moment to moment flow during the time that flow is actually being measured, an advantage not possessed by any other known method. The rotameter is so simple in operation that it should also serve a very useful purpose for the measurement of blood flows in student experiments in the classroom for which as yet no simple and reliable method has been available.

13537

Histamine Release in the Allergic Skin Reaction.*

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It has recently been shown that blood cells from an allergic individual release part of their histamine into the plasma when they are exposed to the allergen *in vitro*.[1] This experiment on humans confirmed previous findings on blood from animals sensitized to egg-white.[2, 3, 4] Furthermore, it helped to substantiate the assumptions

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1 Katz, G., and Cohen, S., *J. A. M. A.*, 1941, **117**, 1782.

2 Katz, G., *Science*, 1940, **91**, 2357.

3 Katz, G., *J. Pharm. and Exp. Therap.*, 1941, **72**, 22.

4 Dragstedt, C., Ramirez de Arellano, M., and Lawton, A. H., *Science*, 1940, **91**, 617.

of the majority of earlier workers that histamine plays a rôle in the symptom complex of the allergic reaction.[5] While it was pointed out in the report referred to above that under certain conditions and to a certain degree such a release of histamine from circulating blood may contribute to the picture of the allergic attack, it may be assumed that histamine originating from the cells of the allergic "shock organs" (chiefly skin and mucous membranes) may take the major part in the humoral processes in allergy. The following is a report on a series of experiments in which an attempt was made to demonstrate that histamine is released in the allergic skin reaction.

In order to obtain tissue fluid for histamine determinations from parts of the skin exposed to allergen, an approach was employed which has been used by some authors for studies on cellular reactions in infectious diseases: production of the cantharides blister.[6] Application of cantharides to the human skin causes an exudation of tissue fluid between the stratum malpighii and the stratum corneum, which presents itself after a certain period of time as a well-defined blister with a minimum or absence of such inflammatory reactions as pain and hyperemia. In the experiments on allergic humans presented in this paper, the histamine content of fluid covering the stratum malpighii after application of cantharides was determined before and after introduction of the allergen.

Methods. The experiments were performed on volunteer patients with clinical ragweed allergy, none of whom had a history of serious allergic reactions, such as attacks of asthma. The dose of ragweed extract that produced a satisfactory skin reaction by intradermal injection was determined in each case. A cantharides plaster of from 2 to 4 cm in diameter was placed on the chest or upper arm and was removed after 15 to 24 hours. In the final method, which was adopted after some preliminary experimentation, the dead skin over the blister was removed. An inverted funnel, with the stem cut off, was then fastened over the denuded area by means of a holder which had previously been fastened to the intact skin with collodion. The funnel was filled with sterile saline, and, by means of 2 screws, pressure was applied from the holder to a degree which prevented fluid from leaking out and did not compress the skin more than necessary. The skin was warmed to 35°C (± 0.5) by means of an infrared lamp. After flushing the funnel-covered tissue several times with saline by means of a syringe and needle, Locke's solution (0.8 to 1.0 cc) was introduced into the funnel and withdrawn and re-

[5] Feldberg, W., *Ann. Rev. Physiol.*, 1941, **3**, 671.

[6] Kaufmann, F., *Klin. Wschnschr.*, 1928, **7**, 1309.

placed with fresh fluid after constant contact periods.

The samples thus obtained were either assayed directly on the isolated, atropinized guinea pig's ileum, or after extraction according to Barsoum-Gaddum and Code.[7] After a control period ragweed extract in saline was injected with a fine needle into the denuded area, in volumes of 0.03-0.05 and the saline samples were continued to be collected. A few control experiments showed that injection of this fluid volume *per se* does not lead to a detectable histamine release into the saline.

Results. Since the results were clearcut, and since the dose of the allergen producing these results usually caused some systemic reactions, only 14 experiments on 9 patients were done. The histamine content of the saline before application of ragweed was in some experiments below the threshold of the assay, and in the others slightly above it and decreasing in successive samples. The observation that tissues which are incubated with saline lose their extracellular histamine rapidly has been discussed in an earlier paper.[8]

In all experiments in which a strong reaction in the denuded area was produced by the ragweed extract, histamine in appreciable amounts was found in the saline. Within the first 10 minutes after introduction of the allergen, the histamine content of the fluid began to rise; it reached its maximum during the first 30 minutes, and it fell to or below the original level within 60 minutes. Roughly parallel with the increase in histamine in the fluid there was a considerable rise in protein as shown by trichloracetic acid precipitation. In three experiments it was determined quantitatively with the photoelectric colorimeter (Klett-Summerson). The results of a representative experiment are shown in Fig. 1, in which the amounts of histamine and protein released from one cm^2 of the denuded area before and after local injection of ragweed extract are recorded. The subject in this experiment suffers from ragweed hayfever for which he has not been treated. He had given a 3 plus skin reaction when tested intradermally with 1:10,000 ragweed extract a few days previous to the experiment. He received 10 times the above concentration and did not consider the ensuing systemic reactions particularly disagreeable. These consisted in an edema of the upper lids and throat irritation, which began 30 minutes after introduction of the allergen, and which were checked with epinephrine at the end of the experiment.

In 2 patients who had been receiving hyposensitizing treatment

[7] Code, C., *J. Physiol.*, 1937, **89**, 257.

[8] Katz, G., *Am. J. Physiol.*, 1940, **129**, 735.

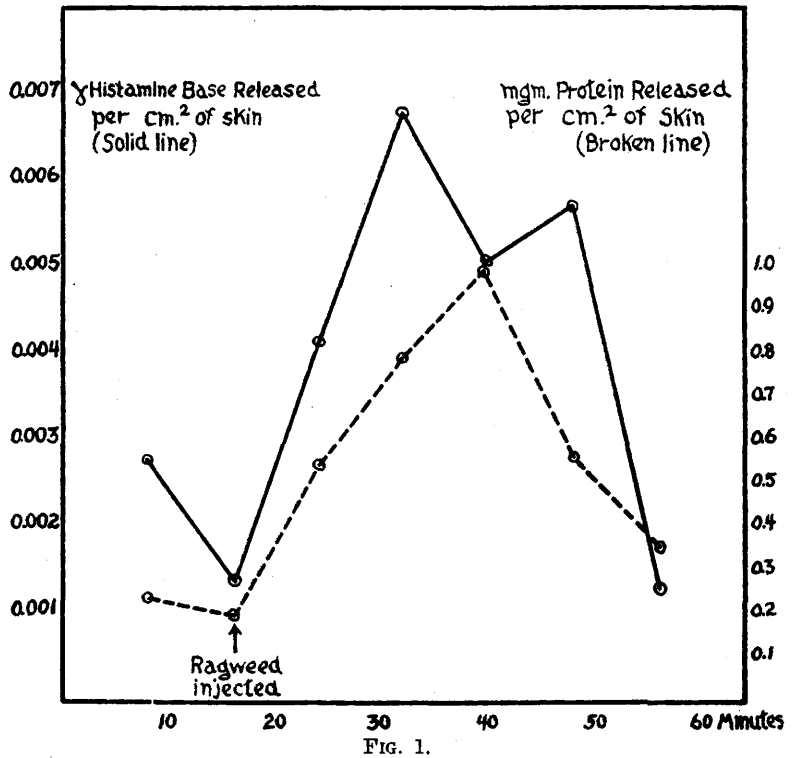

FIG. 1.

previous to the experiments, no local reaction was produced by injection of the allergen in a concentration equal to that with which they were treated at that time. Likewise, no histamine release was found. In 2 other, untreated, cases no histamine release was observed when the ragweed concentration was too small to cause a skin reaction.

Incidental Observations. In all instances in which a local skin reaction was produced, an increased fluid exudation from the denuded area was observed. Its maximum seemed to coincide with the time of the maximum histamine release. Its extent varied in different experiments. This aspect of the allergic skin reaction is being investigated with an improved technic. As in the previous experiments on histamine release in anaphylaxis,[3] the presence of a principle apparently with the properties of the "slow reacting substance" of Feldberg and Kellaway[9] was noticed. It caused a slow, delayed, contraction of the atropinized guinea pig's ileum, temporarily diminished the histamine sensitivity of this organ and was not completely eliminated by the chemical extraction process. How-

[9] Feldberg, W., and Kellaway, C. H., *J. Physiol.*, 1938, **94**, 187.

ever, since this substance was occasionally also found in small quantities in the fluid samples obtained before introducing the allergen, it is not clear whether or not it is a specific product of the allergic cell reaction. It might conceivably partly arise from the cell injury caused by cantharides.

In a series of experiments an attempt to demonstrate a histamine release from the conjunctiva in positive ophthalmic tests was made. No increase in histamine was found in the tear fluid (obtained by blowing mentholated air against the eye) during the conjunctival hyperemia produced by ragweed. If we assume that this reaction is also histaminergic, the failure of these experiments may be due to the technical difficulties of obtaining tear fluid which has been long enough in contact with the tissue, or to a low permeability of the conjunctival membranes to histamine.

Discussion. These experiments show that in the local allergic reaction a substance with the properties of histamine is released from the skin, after the stratum corneum had been separated from the deeper layers through the action of cantharides. From these observations, it seems permissible to assume that this histamine release is likely to occur also in the allergic response of completely intact skin. The technic employed does not allow one to draw more than general conclusions concerning strength and duration of this histamine release, since the amounts of this substance appearing in the saline do not represent the amounts actually released in the deeper layers of the tissue. The rate of diffusion of histamine and perhaps also its inactivation in the skin interfere, possibly to a large extent, with getting an accurate picture of intensity and duration of the reaction in all parts of the skin involved.

The skin histamine release in concentrations which are pharmacologically active doubtlessly contributes, by producing the triple response, to the local change in skin function in the allergic reaction. However, as has been pointed out before, histamine is an important but not the only humoral agent resulting from antigen-antibody union.[3, 5] The liberation of other substances, such as acetylcholine, potassium, and the "slow reacting substance" seem worthy of investigation with the method described in this report.

The increased protein content of the saline during the allergic skin reaction probably had its origin in the well-known increased permeability of the capillaries. Here, as in other aspects of the allergic tissue response, it is not yet clear whether one is dealing with a primary action of the allergen upon the capillary walls, or with the action of histamine released from certain adjacent cells.

It seems probable that an interaction of both of these factors plays a rôle in most allergic tissue reactions.

The failure to obtain a release of histamine in assayable quantities in 2 patients previously treated with hyposensitizing ragweed injections tends to substantiate clinical observations that this treatment chiefly raises the threshold for the amount of allergen necessary to cause a histamine release, and not the threshold to released histamine.

Summary. Cantharides blisters were produced on the skin of allergic human subjects. After removing the stratum corneum over the blisters, the denuded areas were covered with saline, and the release of histamine and protein into the saline was determined before and after production of local allergic reactions by injection of antigen. Both the histamine and the protein content of the saline rose sharply within a few minutes after injection of the allergen and fell to the original level within 60 minutes. An increase in exudation of fluid and the appearance of a substance similar to the "slow reacting substance" were also observed.

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Acute Toxicity of Propionin in Rats and Mice.*

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Propionin is easily hydrolyzed by bacteria[1] and presumably also by the mammalian digestive tract; its toxicity may therefore be due to the effects of propionic acid or soluble propionates. Thus, Ozaki[2] reported that rats ingesting daily about 2.5 g of propionin per kg body weight died in 1 to 3 days. However, when rats were given about 1.4 g of propionin per kg per day, the only toxic effect was a marked regression of growth. Since these findings represent the current knowledge of the toxicity of propionin, this report is presented, not as an exhaustive study but to supply additional information on the acute toxicity of this compound.

Acute Toxicity for Mice. The detailed procedure will be given

* This work was supported in part by a grant from the Carnegie Corporation of New York.

1 Collins, M. A., and Hammer, B. W., *J. Bact.*, 1934, **27**, 473.

2 Ozaki, J., *Biochem. Z.*, 1926, **177**, 156.