

posure to a given carcinogenic agent than is adult subcutaneous tissue in the "static state" of a fully developed rat exposed to a similar dose of the same agent.

Thus, as in the case of epidermoid carcinoma produced by tar and sarcoma produced by the subcutaneous injection of dibenzanthracene and benzpyrene, the age of the animal bears little or no relationship to the latent period in the production of sarcoma by subcutaneous injection of methylcholanthrene.

9263

A Sensitive Method for Quantitative Estimation of Epinephrine in Blood.

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Meltzer and Auer¹ observed that epinephrine causes dilatation of the pupil much more readily after the corresponding superior cervical ganglion has been excised, in rabbits. By means of the "paradoxical" pupil reaction, Joseph and Meltzer² demonstrated liberation of epinephrine from the adrenals on stimulation of the splanchnic nerves. This was confirmed by Elliott,³ who found that dilatation of the pupil and retraction of the nictitating membrane, resulting from electrical excitation of the splanchnic nerve, do not occur in the absence of the adrenals.

Following these qualitative observations, Stewart and Rogoff⁴ employed the eye reactions (pupil dilatation, retraction of nictitating membrane and widening of palpebral aperture), sensitized by corresponding superior cervical ganglionectomy, for quantitative studies on the epinephrine output of the adrenals. A method of "auto-assay" was devised based upon reactions of the sensitized eye when adrenal vein blood was collected in a "cava pocket" for a given time and then released into the circulation. The intensity of the epinephrine reactions was measured by intravenous injections of appropriate amounts of epinephrine.

In our experience with various biological test objects used for

* Aided by the G. N. Stewart Memorial Fund and a grant from the Rosenwald Family Association.

¹ Meltzer, S. J., and Auer, C. M., *Am. J. Physiol.*, 1904, **11**, 28.

² Joseph, D. R., and Meltzer, S. J., *Am. J. Physiol.*, (Proc. Am. Physiol. Soc.), 1912, **20**, xxxiv.

³ Elliott, T. R., *J. Physiol.*, 1912, **44**, 374.

⁴ Stewart, G. N., and Rogoff, J. M., *J. Pharm. Exp. Therap.*, 1916, **8**, 479.

detecting epinephrine, it was observed that sensitivity of the reagent usually increases in the course of an experiment. An increase of 5 to 25 or more times may occur after the test object has been used for an hour or two. This is quite common with segments of rabbit's intestine and uterus. In earlier work we observed increased sensitivity to epinephrine with the so-called "denervated" or sensitized eye reactions but not of such magnitude as was seen with intestine segments.

The present report will show that it is possible to increase sensitivity of the denervated eye to epinephrine to such a degree that the reactions can be utilized for detection of epinephrine in small amounts in blood-serum or in saline solutions. Quantitative estimation of the epinephrine concentration in the liquid can be made by comparing the reactions with those obtained by injection of epinephrine solutions of different concentrations. By this method it was found possible to detect, with certainty, epinephrine in serum or in physiological saline solution in dilutions of 1:100,000,000 to 1:500,000,000. Sometimes the sensitivity is not so great and occasionally an animal may yield reactions with even higher dilutions.

Procedure. The method depends upon further sensitizing the eye to epinephrine after it has already been sensitized by superior cervical ganglionectomy. This is accomplished by repeated injections of epinephrine in amounts sufficient to elicit small but definite reactions. Cats were used 5 or 6 days to 2 weeks after unilateral excision of the superior cervical ganglion. Sensitive reactions have been obtained up to about a month after ganglionectomy. The animal is anesthetized (urethane, 0.75 to 1.0 gm. per kg. body weight by stomach tube). A portion of the femoral or external jugular vein is exposed and a loose ligature is adjusted to facilitate injection into the vein. The carotid artery on the side of the sensitized eye is similarly prepared. A syringe with a very small hypodermic needle is used for the injections.

Epinephrine in saline solution is injected intravenously at intervals of about 5 minutes. When it is observed that the reacting structures are becoming more sensitive the injections are made directly into the carotid artery on the side of the sensitized eye. The injections are repeated, as in the case of the intravenous injections, until sensitivity of the reacting structures is markedly increased. When this occurs the animal can be used for assay of an unknown which contains an epinephrine concentration within the range of sensitivity of the test object.

Quantities ranging from 0.1 cc. to 1.0 cc. of the epinephrine solutions are used, usually beginning with 0.25 cc., 0.5 cc. and 1.0 cc.

in the intravenous injections. It is rarely necessary to use more than 0.5 cc. quantities in the carotid artery injections. Concentrations of epinephrine should be such as to yield small but unequivocal reactions. In the beginning it usually requires about 0.5 cc. of 1:1,000,000 to 1:2,000,000 to cause a distinct reaction when injected intravenously. At this stage reactions of the same intensity are produced by solutions of epinephrine 10 to 20 times as dilute, if injected into the carotid artery. A reaction obtained by injecting a given amount of epinephrine solution in a certain concentration is obtained with half that amount if the concentration is double or with double the amount if the concentration is half the original.

A much greater increase in sensitivity is ultimately produced by direct injections into the carotid artery than by the intravenous injections. With injections of 0.2 cc. to 0.5 cc. quantities, epinephrine concentrations of 1:100,000,000 to 1:200,000,000 commonly can be detected with certainty; sometimes concentrations of 1:300,000,000 to 1:500,000,000 have been detected and in one case definite reactions were obtained with solutions containing 1:1,600,000,000 epinephrine. The maximum sensitivity lasts for a while and then gradually diminishes. Quantitative determinations should be made as soon as the increase is large enough. They can be repeated if further increase in sensitivity occurs.

Usually the iris is the most sensitive of the reacting structures. Sometimes, however, the nictitating membrane reacts more readily and the widening of the palpebral aperture is also more definite than the pupil dilatation. This is often the case when the pupil is already considerably dilated before the epinephrine is injected. In a good reaction all of the structures participate. It should be remembered that substances other than epinephrine might occur in some blood specimens which may cause dilatation of the sensitized pupil. In this respect the intestine and uterus method is more reliable than the method under discussion. We have found, in one instance, that systemic blood-serum obtained from an animal in poor condition and under asphyxia caused a small pupil reaction which, however, was not difficult to distinguish from a larger epinephrine reaction.

The reaction to epinephrine in serum is the same as when the dilutions are made in saline solution. In case of intra-arterial injections the reactions occur in about 2 to 4 seconds; with intravenous injection they appear in about 6 to 15 seconds, according to the condition of the circulation in the animal. It is important that the rate of injection be constant in all observations. Especially in case of the intra-arterial injections, is it necessary to avoid sudden in-

jection since the sudden increase in pressure may result in a spurious dilatation of the pupil. On the other hand, if the injection is too slow the reaction may fail, particularly when working with high dilutions of epinephrine. It should be aimed to introduce the material at about the same rate as that of the blood flow in the blood vessel. A mechanical device has been found useful for this purpose.

We have compared the results of assay of epinephrine in serum of adrenal vein blood and of systemic blood to which epinephrine was added, using this method and the rabbit intestine method as employed by Stewart and Rogoff. The results obtained by the two methods compared remarkably well in practically every instance.

Since it is known that the epinephrine in blood is contained in the plasma or serum,⁵ it is possible, by using serum for the tests, to study problems related to hyperepinephremia alleged to exist in various diseases. Any concentration of epinephrine found in the serum would correspond to about half that concentration in the whole blood. Thus, the test object, when sensitized to the degree found possible in the present investigation, could detect epinephrine in the systemic circulation if hyperepinephremia exists. Studies on the relation of the adrenals to hypertension and on the existence of detectable quantities of epinephrine in the circulation are in progress.

9264 P

Use of Air in Basal Metabolism.

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In the past there has been no reference in the literature to the use of air instead of oxygen in the semi-portable type of basal metabolimeters which are commonly used clinically. Yet, obviously, there may be certain advantages to using air instead of oxygen in a clinical metabolimeter. An article describing an air-using machine has just been published in a South American periodical by a doctor in Mexico City.¹ In certain parts of the world, oxygen is expensive and difficult to obtain.

It has been definitely shown² that the oxygen tension of the air

⁵ Stewart, G. N., and Rogoff, J. M., *J. Pharm. Exp. Therap.*, 1917, **9**, 393.

¹ Macías, F. I., *Semana méd.*, 1936, **1**, 680.

² "Medical Studies on Aviation," *J. Am. Med. Assn.*, 1918, **71**, 17, 1383.