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B. T. GALLOWAY, *Chief of Bureau.*

THE USE OF SUPRARENAL GLANDS IN THE PHYSIOLOGICAL TESTING OF DRUG PLANTS.

BY

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., May 24, 1907.

Sir: I have the honor to transmit herewith, and to recommend for publication as Bulletin No. 112 of the series of this Bureau, the accompanying technical manuscript entitled "The Use of Suprarenal Glands in the Physiological Testing of Drug Plants." This paper was prepared by Dr. Albert C. Crawford, Pharmacologist in Drug-Plant Investigations, as one of a series of publications on the subject of drug testing, and has been submitted by Dr. Rodney H. True, Physiologist in Charge, with a view to its publication.

This paper is preliminary to a consideration of the subject of the testing of ergot, one of the most valuable and variable of vegetable drugs. It has been proposed by recent investigators that the most acceptable means of measuring the activity of ergot is to standardize it against a known preparation of the active principle of the suprarenal glands. In order, therefore, to enable us to carry out the ergot test, the presentation of a means of standardizing the active principle of suprarenal glands is a preliminary step.

Among the great advance steps taken by medicine in later years, the attempt to bring medicinal agents to a known and, when possible, uniform standard of action is one of the most important. Many drugs are now standardized by chemical methods and can be administered by the physician in full confidence that his remedy is capable of exerting the desired degree of action. In the case of others in which the active principles are not as yet known or in which the principle will not admit of isolation, testing by physiological means has come to be recognized as a prime necessity. Since this phase of drug investigations is still young, a considerable diversity in methods exists. It is hoped that this paper, which treats of methods of testing the active principle of suprarenal glands, may contribute toward greater uniformity in this important matter and make more generally available than is now the case the essentials of an important means whereby physiology may serve medicine.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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THE USE OF SUPRARENAL GLANDS IN THE PHYSIOLOGICAL TESTING OF DRUG PLANTS.

INTRODUCTION.

It has long been recognized that many of the important pharmacopœal preparations can not be accurately standardized by any known chemical processes, so that for this purpose physiological methods have been employed.[a]

There have been some inquiries as to where information concerning this subject could be obtained, and as most of the data occur in sources which are not usually accessible it was deemed wise to abstract this literature and present the methods in some detail. It must be remembered that as our knowledge increases these tests are sure to suffer change, a fate which has been the lot of chemical assay processes. A few years ago gravimetric assay methods were used almost entirely, but now only when titration methods are unavailable. These methods have become an essential to all analytical pharmaceutical laboratories, and thousands of dollars are spent every year in this country for carrying out these tests.

While often mechanically simple in their execution they require considerable experience to interpret them properly, and for this reason some of the large drug firms err in employing inexperienced persons to perform them.

These tests can be used not only with preparations in which the active principle is little known, but also to control chemical processes where the active principle is well recognized. Thus the writer has controlled the assay for atropin by noting the minimum quantity necessary to cause dilatation of the pupil and standardizing this with a known solution of atropin.

[a] Standardization of pharmaceutical preparations. Brit. Med. Jour., vol. 2, p. 583, 1906.

No one would now think of using any of the aconitins in medicine without first determining their toxicity, whatever the result of the chemical assay.

Kobert has called attention to this increased importance of the pharmacologist and has claimed that these tests should be an essential for all medico-legal cases, and he has shown that the physiological test may respond where the chemical one is not sufficiently delicate.[a]

Certain simple physiological tests, such as the dilatation of the pupil with atropin, the production of a tingling sensation in the tongue by aconite, and the correct tasting of preparations, have been recognized by the Pharmacopœia, and the testing of diphtheria antitoxin has at last obtained recognition.[b]

It must be remembered that animals respond differently according to various conditions. Thus Dixon[c] has cited the influence of cerebral development in animals as influencing the response to cocain, as follows:

Animal.	Grams of brain per kilo of animal.	Dose of cocain per kilo of animal necessary to produce convulsions.
Rabbit	4	0.18
Guinea pig	7	.07
Pigeon	8	.06
Dog	9	.02
Ape	18	.012

Various other illustrations could be given, so that the animals in most cases should correspond as nearly as possible in species, sex, age, and weight.

SUPRARENAL GLANDS.

SEPARATION OF THE ACTIVE PRINCIPLE.

Attention was recently called to the marked blood-pressure-raising properties of the suprarenal glands in the work of Oliver and Schaefer,[d]

[a] Kobert, R. Ueber d. Bedeutung d. biologisch. Giftnachweis f. d. gerichtl. Med. Ber. d. Deutsch. Pharm. Gesells., vol. 13, p. 325, 1903.
Scholtz, K. Wertbestimmung d. Jequiritols u. d. Jequiritol-Heilserums durch Tierexperimente. Arch. f. Augenheilkunde, vol. 55, p. 209, 1906.

[b] Otto, R. Die staatliche Prüfung d. Heilsera. Arbeit. a. d. Königl. Institut f. Exper. Therapie z. Frankfurt, 1906.

[c] Dixon, W. E. Bio-chemical standardization of drugs. Pharm. Jour., vol. 75, p. 156, 1905.

[d] Oliver, G., and Schaefer, E. A. Physiological effects of extracts of suprarenal capsules. Jour. Physiol., vol. 18, p. 230, 1895.
On the physiological action of extract of the suprarenal capsules. Proc. Physiol. Soc., p. i, Jour. Physiol., vol. 16, 1894; Proc. Physiol. Soc., p. ix, Jour. Physiol., vol. 17, 1894–95.

and later by Cybulski, Szymonowicz, Boruttau, and others.[a] Vulpian[b] had in 1856 noted the presence in them of certain principles giving peculiar color reactions, and from this time these color reactions were believed to be due to the presence of pyrocatechin or a derivative of it.[c]

The chemical work—at least that which has been done in this country—has been carried out mainly from the influence of Professor Abel's laboratory. Abel himself isolated a body to which he gave the name epinephrin, and calculated the empirical formula to be $C_{17}H_{15}NO_4$, but he was compelled to change this to $C_{10}H_{11}NO_3$ by the withdrawal of one benzoyl group, and later to $C_{10}H_{13}NO_3\frac{1}{2}H_2O$.[d]

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[a] Szymonowicz, L. Die Function d. Nebenniere. Arch. f. Gesam. Physiol., vol. 64, p. 97, 1896.

Boruttau, H. Erfahrung. über d. Nebennieren. Arch. f. Gesam. Physiol., vol. 78, p. 97. 1899.

[b] Vulpian, A. Note sur quelques reactions propres à la substance des capsules surrénales. Comp. Rend. Acad. des Sci., vol. 43, p. 663, 1856.

Cloez, S., and Vulpian, A. Note sur l'existence des acides hippurique et choléique dans les capsules surrénales des animaux herbivores. Comp. Rend. Acad. des Sci., vol. 45, p. 340, 1857.

[c] Krukenberg, C. F. W. Die farbigen Derivate der Nebennierenchromogene. Arch. f. Path. Anat., vol. 101, p. 542, 1885.

Brunner, H. Zur Chemie d. Lecithine u. d. Brenzcatechins, Bestandtheile der Nebennieren. Schweiz. Woch. f. Chem. u. Pharm., vol. 30, p. 121, 1892.

Mühlmann, M. Zur Physiologie der Nebenniere. Deutsch. Med. Woch., vol. 22, p. 409, 1896.

Fraenkel, S. Beitr. z. Physiol. u. physiol. Chemie d. Nebenniere. Wien. Med. Blätter, vol. 19, pp. 211, 228, 246, 1896.

[d] Abel, J. J., and Crawford, A. C. On the blood-pressure-raising constituent of the suprarenal capsule. Johns Hopkins Hospital Bul., vol. 8, p. 151, 1897.

Abel, J. J. Ueber den blutdruckerregenden Bestandtheil d. Nebenniere, das Epinephrin. Zeits. f. Physiol. Chemie, vol. 28, p. 318, 1899.

Further observations on epinephrin. Johns Hopkins Hospital Bul., vol. 12, p. 80, 1901.

On epinephrin and its compounds. Amer. Jour. Pharm., vol. 75, p. 301, 1903.

Weitere Mittheil. ü. d. Epinephrin. Ber. d. Deutsch. Chem. Gesells., vol. 36, p. 1839, 1903.

The function of the suprarenal glands. Contrib. to Med. Research, dedicated to V. C. Vaughan, 1903, p. 138.

On the phenylcarbamic esters of epinephrin. Proc. Amer. Physiol. Soc., 1899, p. xvii, Amer. Jour. Physiol., vol. 3, 1900.

On a simple method of preparing epinephrin and its compounds. Johns Hopkins Hospital Bul., vol. 13, p. 29, 1902.

Abel, J. J., and Taveau, R. de M. On the decomposition products of epinephrin hydrate. Jour. Biol. Chem., vol. 1, p. 1, 1905.

NOTE.—Full literature on the suprarenals may be found in Möller, S., Kritisch-exper. Beitr. z. Wirkung d. Nebennierenextraktes, Dissert., Berlin, 1906.

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Von Fürth[a] obtained a principle which he named suprarenin and gave the formula $C_5H_9NO_2$ or $C_5H_7NO_2$, but later changed this to $C_9H_{13}NO_3$.

Takamine[b] simplified the method of isolation and made it commercially available, giving his preparation the name adrenalin, with the formula $C_{10}H_{15}NO_3$.

Simultaneous with Takamine's paper, Aldrich, Abel's former associate, published his results.[c] His body was evidently much purer than Takamine's, as he purified before precipitating, but his method was not commercially available on account of the necessary purification from the lead. Aldrich adopted Takamine's name adrenalin, although his formula $C_9H_{13}NO_3$ differed by CH_2 from that of Takamine. These two preparations are often confused. Aldrich pointed out that if the benzoyl group was removed from Abel's original formula, the resultant formula was close to his. All three investigators—Abel, Takamine, and Aldrich—were dealing with the same body, but in varying degrees of purity.

Abel has compared the analytical data furnished by Aldrich and Takamine, and declares that the analyses do not bear out the empirical formulæ deduced.[d] The formula of Aldrich has been corroborated by Bertrand in France,[e] and adopted by Pauly, von Fürth, Stolz, Abderhalden, and Bergell, in Germany.[f] The two latter investigators used Abel's purification method, but came to different conclu-

[a] Von Fürth, O. Zur Kenntniss d. brenzkatechinähnlich. Substanz in d. Nebennieren. Zeits. f. Physiol. Chemie, vol. 24, p. 142, 1898; vol. 26, p. 15, 1898–99; vol. 29, p. 105, 1900.

Zur Kenntniss des Suprarenins. Beitr. z. Chem. Phys. u. Path., vol. 1, p. 243, 1902.

Zur Kenntniss des Suprarenins (Adrenalins). Sitz. d. Kaiserl. Akad. d. Wissen. Wien, Math.-natur. Kl., vol. 112, pt. 3, 1903.

Zur Kenntniss des Suprarenins (Adrenalins). Monats. f. Chem., vol. 24, pp. 261–290, 1903.

[b] Takamine, J. Adrenalin, the active principle of the suprarenal glands. Amer. Jour. Pharm., vol. 73, p. 523, 1901.

The blood-pressure-raising principle of the suprarenal glands. Therap. Gaz., vol. 25, p. 221, 1901.

[c] Aldrich, T. B. Preliminary report on the active principle of the suprarenal gland. Amer. Jour. Physiol., vol. 5, p. 457, 1901.

Adrenalin, the active principle of the suprarenal glands. Jour. Amer. Chem. Soc., vol. 27, p. 1074, 1905.

[d] Abel, J. J. On epinephrin and its compounds. Amer. Jour. Pharm., vol. 75, p. 309, 1903.

[e] Bertrand, G. Sur la composition chimique et la formule de l'adrénaline. Comp. Rend. Acad. d. Sci., vol. 139, p. 502, 1904.

[f] Pauly, H. Zur Kenntniss des Adrenalins. Ber. d. Deutsch. Chem. Gesells., vol. 36, pt. 3, p. 2944, 1903; vol. 37, pt. 2, p. 1388, 1904.

Abderhalden, C., and Bergell, P. Zur Kenntniss d. Epinephrins. Ber. d. Deutsch. Chem. Gesells., vol. 37, pt. 2, p. 2022, 1904.

Ueber d. Epinephrin. Münch. Med. Woch., vol. 51, p. 1003, 1904.

sions from Abel. It was also adopted by Jowett and by Barger and Ewins in England.[a] These latter authors are especially emphatic in support of Aldrich's formula. These differences in results have not yet been finally adjusted.[b] The difficulty may be due to the fact that there is in adrenalin a series of chemically similar bodies,[c] as it is well known that blood-pressure-raising properties and the chemical reactions shown by adrenalin are given by other pyrocatechin derivatives.[d]

The active principle resides largely in the medullary portion of the suprarenal glands, although the cortex also contains some.[e] Accessory suprarenal glands which are found in various portions of the abdominal cavity also contain principles having blood-pressure-raising properties. Blood-pressure-raising principles are also claimed to be present in other organs, pituitary bodies, etc.[f]

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[a] Jowett, H. A. D. The constitution of epinephrin. Jour. Chem. Soc. Trans., vol. 85, p. 192, 1904.

Barger, G., and Ewins, A. J. Note on the molecular weight of epinephrin. Chem. News, vol. 93, p. 90, 1906.

Note.—For a review of the relation of the early chemical workers, see Maben, T., Adrenalin: the active principle of the suprarenal gland, in Pharm. Jour., 1907, p. 388. A reply to Maben is found in Martin, W., Epinephrin or adrenalin, in Pharm. Jour., 1907, p. 447.

[b] Aldrich, T. B. Is adrenalin the active principle of the suprarenal gland? Amer. Jour. Physiol., vol. 7, p. 359, 1902.

[c] Halle, W. L. Ueber d. Bildung d. Adrenalins im Organismus. Beitr. z. Chem. Physiol. u. Pathol., vol. 8, p. 277, 1906.

Elliott, T. Action of adrenalin. Jour. Physiol., vol. 32, p. 462, 1905. Elliott writes as follows: "By bubbling oxygen through adrenalin solution (Parke-Davis's 0.1% HCl solution, diluted to 1:2,000 and exactly neutralised with Na_2CO_3) I obtained a brown liquid which contained no adrenalin, but was fairly potent to cause vaso-constriction. In this respect it had one-twentieth of the power of adrenalin. But even 4 c. c. of the solution, which correspond to an original content of 2 mgm. adrenalin, and in respect of ability to raise blood pressure to 0.1 mgm. of adrenalin, caused no movements of iris or nictitating membrane. In the same test cat 0.03 mgm. adrenalin gave maximal rise of blood pressure and typical eye movements. Four c. c. of the oxydised solution were then injected beneath the skin of a rabbit, and caused neither glycosuria nor prostration."

Moore B., and Purington, C. On the chromogen of the suprarenal medulla. Proc. Amer. Physiol. Soc., 1899, p. xvi; Amer. Jour. Physiol., vol. 3, 1900.

[d] Dakin, H. D. On the physiological activity of substances indirectly related to adrenalin. Proc. Royal Soc. London, ser. B, vol. 76, p. 498, 1905.

[e] Salvioli, I., and Pezzolini, P. Sur le différent mode d'agir des extraits médullaire et cortical des capsules surrénales. Arch. Ital. de Biol. vol. 37, p. 380, 1902.

[f] Schmid, J. Ueber d. blutdrucksteigernde Substanz d. Niere. Med. Klinik, 1906, p. 976.

Livon, C. Sécrétions internes: Glandes hypertensives. Comp. Rend. Hebd. Soc. de Biol., vol. 50, p. 98, 1898.

Brown, O. H., and Joseph, D. R. Effects of intravenous injections of extracts of the bone marrow. Amer. Jour. Physiol., vol. 16, p. 110, 1906.

COLOR TESTS.

Adrenalin is described as a microcrystalline powder which possesses basic properties and acts as a reducing agent. Strictly speaking, this body can not be classed with the alkaloids.

Freshly prepared solutions are colorless, but become rose colored under the influence of light and air, especially in dilute solutions. After long standing these solutions finally become brown and lose their activity. This oxidation especially occurs in an alkaline medium, so that commercial solutions are usually made acid.[a]

The active principle when in solution gives a green color on the addition of ferric chlorid. This green passes into a purple and finally into a carmine on the addition of ammonia. A dilute solution of iodin turns adrenalin solution to a rose color.

This production of a green color with ferric chlorid has been utilized by Battelli[b] as a method of estimating the amount of active principle present. The formation of this color is, however, influenced by acidity and appears badly in very acid solutions,[c] but the main difficulty with this method is that the delicate green is hard to recognize in great dilution.

Cameron[d] states that this is a rough method for assaying solutions of over 1–40,000 and that it fails for brown or solutions more dilute than 1–40,000.

Von Fürth[e] has also proposed a color test with iron chlorid, sodium carbonate, and potassium sodium–tartrate, but this does not offer any advantages and is subject to the same criticism as other color reactions.

Abelous, Soulié, and Toujan[f] have proposed using the iodin reaction for the same purpose, judging not by the amount of iodin used but by the shade of rose color produced. This is ascertained by con-

[a] Livon, C. Action des vieilles solutions d'adrénaline. Comp. Rend. Hebd. Soc. de Biol., vol. 1, p. 125, 1904.

Battelli, F. Transformation de l'adrénaline "in vitro." Comp. Rend. Hebd. Soc. de Biol., vol. 54, p. 1435, 1902.

[b] Battelli, F. Dosage colorimétrique de la substance active des capsules surrénales. Comp. Rend. Hebd. Soc. de Biol., vol. 54, p. 571, 1902.

[c] Boulud, R., and Fayol. Sur le dosage colorimétrique de l'adrénaline. Comp. Rend. Hebd. Soc. de Biol., vol. 55, p. 358, 1903.

[d] Cameron, I. D. On the methods of standardising suprarenal preparations. Proc. Roy. Soc. Edinburgh, vol. 26, p. 157, 1906.

[e] Von Fürth, O. Zur Kenntniss der brenzcatechinähnlich. Substanz d. Nebennieren. Zeits. f. Physiol. Chem., vol. 29, p. 115, 1900.

Zur Kenntniss des Suprarenins. Beitr. z. Chem. Physiol. u. Path., vol. 1, p. 244, 1902.

[f] Abelous, J. E., Soulié, A., and Toujan, G. Dosage colorimétrique par l'iode de l'adrénaline. Comp. Rend. Hebd. Soc. de Biol., vol. 1, p. 301, 1905.

Sur un procédé de contrôle des dosages chimique et physiologique de l'adrénaline. Comp. Rend. Hebd. Soc. de Biol., vol. 60, p. 174, 1906.

trasting it with a standard solution of adrenalin freshly prepared which has also been treated with iodin. By this method they claim that the suprarenal glands (sheep) contain 1.47 mg. in every gram, while they state that Batelli by his method found 1.45 mg.[a]

Details of this method can be found in English in C. E. Vandekleed's "Method for the preparation of the active principle of the suprarenal gland" (Pharmaceutical Era, 1906, vol. 36, p. 478).

PRINCIPAL PHYSIOLOGICAL TESTS.

Toujan,[b] one of the originators of the iodin color test, states that the physiological response is more delicate than the chemical, although he objects to the former as being too inconstant for vigorous results. One of the difficulties with color reactions as a quantitative test is the fact that nothing is known as to whether in decomposition of the active principle the change in color reactions runs parallel with the loss in blood-pressure-raising properties; in fact, the evidence rather makes this doubtful. Battelli claims that certain suprarenal glands on removal from the body lose their reaction to iron chlorid, but retain their blood-pressure-raising properties.[c] When injected into the vein of an animal an extract of these glands or a solution of the active principle will cause a marked rise in the general blood pressure associated with a temporary slowing of the heart, owing to an action on the vagus centers, but if the vagi are cut or atropin is given before the injection of the suprarenal preparation, instead of the cardiac slowing there will be a marked acceleration of the heart beats and the maximum blood pressure effect will be obtained.[d] This rise in blood pressure is mainly due to a local action on the small blood vessels, or rather on the sympathetic nerve terminals in their walls.[e] In fact, Elliott considers that a "positive reaction with adrenalin is a trustworthy proof of the existence and nature of sympathetic nerves in any organ."

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[a] Compare Battelli, F., and Ornstein, S. La suppléance des capsules surrénales au point de vue de leur richesse en adrenaline. Comp. Rend. Hebd. Soc. de Biol., vol. 61, p. 677, 1906.

[b] Toujan, G. Recherches expér. sur l'adrénaline. Thèse, Toulouse, 1905, p. 40.

[c] Ornstein, S. Suppléance des capsules surrénales. Thèse, Genève, 1906, p. 12.

[d] Elliott, T. R. Action of adrenalin. Jour. Physiol., vol. 32, p. 447, 1905.
Mathieu, X. Action de l'adrénaline sur le coeur. Jour. de Physiol. et de Path. Gen., vol. 6, p. 435, 1904.
Kahn, R. H. Beob. über d. Wirkung d. Nebennierenextractes. Arch. f. Anat. u. Physiol., Physiol. Abtheil., 1903, p. 522.
Plumier, L. Action de l'adrénaline sur le circulation cardiopulmonaire. Jour. de Physiol. et de Path. Gen., vol. 6, p. 655, 1904.

[e] Brodie, T. G., and Dixon, W. E. Contributions to the physiology of the lungs. Jour. Physiol., vol. 30, p. 476, 1904.
See also von Frey. Beitr. z. Kenntniss d. Adrenalinwirkung. Sitzb. d. Phys. Med. Gesells. z. Würzburg, 1905, p. 43:50.

Besides this action on the sympathetic nerve terminals there is some action on the heart itself.[a]

ACTION ON THE EYE.

The action on the small blood vessels is well shown by dropping a dilute solution into the conjunctival sac of an animal, when the conjunctiva becomes pale and bloodless and the pupil dilates.[b] This action on the conjunctiva occurs even after the use of solutions as dilute as 1–120,000. The action on the pupil of the excised frog eye has been advocated by Ehrmann[c] as a method for determining the amount of active principle present in unknown solutions, but the objection to this method is the difficulty in exactly measuring the size of the pupil and the uncertainty as to absorption through the eye membranes. In Ehrmann's hands, 0.000025 mg. could be thus determined with certainty, although 0.0000001 gram produced a distinct dilatation, while 0.00000005 gram was inactive. Cameron's results with this method were unsatisfactory.

ACTION ON ANIMALS.

A second method of showing this vaso-constrictor action is by perfusion of the blood vessels in frogs. Läwen[d] has obtained a response showing a decided constriction of the vessels with 0.2 per million adrenalin, but the method is tedious and frogs vary much in their response. Cameron obtained a feeble reaction with 0.1 per million. Details of this method may be found in English in Cameron's paper. Meyer[e] placed sections of the beef subclavian arteries in Ringer's solution with the addition of adrenalin. He found that most of them responded with under 1-100,000,000, but not all. With increasing amounts of adrenalin the contraction was greater—usually 1-50,000

[a] Gottlieb, R. Ueber d. Wirkung d. Nebennierenextracte auf Herz u. Blutdruck. Arch. f. Exp. Path., vol. 38, p. 99, 1897.

[b] Meltzer, S. J., and Auer, K. M. Ueber d. Einfluss d. Nebennierenextraktes auf d. Pupille d. Frosches. Cent. f. Physiol., vol. 18, p. 317, 1904.

Kahn, R. H. Ueber d. Beeinflussung d. Augendruckes durch Extrakte chromaffinen Gewebes (Adrenalin). Zent. f. Physiol., vol. 20, p. 33, 1906.

Lewandowsky, M. Ueber d. Wirk. d. Nebennierenextractes auf d. glat. Muskeln, im besond. des Auges. Arch. f. Anat. u. Phys., Physiol. Abtheil., 1899, p. 360.

[c] Ehrmann, R. Ueber eine physiol. Werthbestimmung des Adrenalins. Arch. f. Exper. Path. u. Pharmakol., vol. 53, p. 97, 1905.

Zur Physiol. u. experiment. Path. der Adrenalinsekretion. Arch. f. Exp. Path. u. Pharmakol., vol. 55, p. 39, 1906.

Ueber die Wirkung des Adrenalins auf die Hautdrüsensekretion des Frosches. Arch. f. Exp. Path. u. Pharmakol., vol. 53, p. 137, 1905.

[d] Läwen, A. Quantitative Untersuchungen über d. Gefässwirkung von Suprarenin. Arch. f. Exp. Path. u. Pharmakol., vol. 51, p. 422, 1904.

[e] Meyer, O. B. Ueber einige Eigenschaft. d. Gefässmuskulatur. Zeits. f. Biol., vol. 48, p. 365, 1906.

gave the maximum, although some gave the maximum contraction with 1-100,000. This method has not yet been controlled as a quantitative procedure by any other observer, but deserves investigation.

Various synthetic adrenalins have been made, but while many give the chemical reactions of this body yet they do not have its physiological action.[a] Others have almost the same blood-pressure-raising properties as adrenalin. Thus the compound made by Dakin[b] caused a definite rise in blood pressure in rabbits with cut vagi, when injected in doses of 0.000001 gram.

The field of usefulness for the suprarenal glands in therapy is increasing,[c] but these preparations are being used almost with recklessness. Reports of toxic symptoms and secondary hemorrhage following their use are now appearing.[d]

In the case of animals it has been shown that the repeated intravenous injections of small doses of adrenalin will cause changes in the heart muscle (myocarditis)[e] and degeneration of the arterial walls resembling, if not identical with, arterio-sclerosis.

D'Amato[f] has made the interesting observation that arterial degeneration occurs from repeated use of small doses of suprarenal

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[a] Barger, G., and Jowett, H. A. D. Synthesis of substances allied to epinephrin. Jour. Chem. Soc. Trans., vol. 87, pt. 2, p. 967, 1905.

Stolz, F. Ueber Adrenalin und Alkylaminoacetobrenzcatechin. Ber. d. Deutsch. Chem. Gesells., vol. 37, pt. 4, p. 4149, 1905.

Friedman, E. Konstitution des Adrenalins. Beitr. z. Chem. Physiol. u. Path., vol. 8, p. 95, 1906.

Loewi, O., and Meyer, H. Ueber d. Wirkung synthetischer dem Adrenalin verwandter Stoffe. Arch. f. Exp. Path. u. Pharmakol., vol. 53, p. 213, 1905.

[b] Dakin, H. D. Synthesis of a substance allied to adrenalin. Proc. Roy. Soc. London, ser. B, vol. 76, p. 491, 1905.

On the physiological activity of substances indirectly related to adrenalin. Proc. Roy. Soc. London, ser. B, vol. 76, p. 498, 1905.

[c] Kreuzfuchs, S. Einige Erfahrung. über innere Adrenalindarreichung. Wien. Med. Presse, vol. 47, p. 922, 1906.

Oppenheim, R., and Loeper, M. La médication surrénale. Paris, 1904.

[d] Burnett, C. H. Results of a mistake in putting up a prescription for adrenalin chloride to be used as a nasal spray. Internat. Clinic, vol. 4, p. 25, 1902.

Roberts, L. M. Antidote for suprarenal preparations. Jour. Amer. Med. Assoc., vol. 47, p. 2159, 1906.

Potts, B. H. Danger from the careless use of the alkaloid of the suprarenal gland. Jour. Amer. Med. Assoc., vol. 47, p. 1188, 1906.

[e] Pearce, R. M. Experimental myocarditis: A study of the histological changes following intravenous injections of adrenalin. Jour. Exper. Med., vol. 8, p. 400, 1906.

[f] D'Amato, L. Weitere Untersuch. über d. von den Nebennierenextrakten bewirkten Veränderungen d. Blutgefässe. Berl. Klin. Woch., 1906, pp. 1100, 1131.

Elliott, T. R., and Durham, H. E. On subcutaneous injections of adrenalin. Jour. Physiol., vol. 34, p. 498, 1906.

NOTE.—Data on the effects on the urinary secretion can be found in Bardier, E., and Frenkel, H., Action de l'extrait capsulaire sur la diurèse et la circulation rénale, Jour. de Physiol. et de Path. Gén., vol. 1, p. 950, 1899.

preparations by mouth, which by this method of administration fail to produce any rise in blood pressure. Metabolic changes, shown by the presence of glycosuria, and histological changes, especially in the involuntary muscle fibers of the stomach and intestine, and changes in the liver cells have been noted in experimental work on animals,[a] so that it becomes imperative to exercise more care in the use of these preparations and to determine accurately the amount of the active principle used.

MEASUREMENT OF THE RISE OF BLOOD PRESSURE IN HIGHER ANIMALS.

At present the most satisfactory method of standardizing these preparations is by measuring the actual rise in blood pressure which follows the intravenous injection into animals of definite amounts of a solution of the pure active principle and comparing this rise with that produced by the same amount of the solution to be tested. The method is based on the fact that in the same animal the same amount of the active principle will produce the same rise in blood pressure, provided the conditions are unaltered.[b] The vagi nerves should first be cut or atropin injected to secure the full pressor effects of the injection. These measurements are usually made by

[a] Blum, F. Nebennierendiabetes. Deutsch. Arch. Klin. Med., vol. 71, p. 146, 1901.
Loeper, M., and Crouzon, O. L'action de l'adrénaline sur le sang. Arch. d. Méd. Expér., vol. 16, p. 83, 1904.
Paton, D. N. Effect of adrenalin on sugar and nitrogen excretion in the urine of birds. Jour. Physiol., vol. 32, p. 59, 1905.
Citron, J. Ueber d. durch Suprarenin experiment. erzeugt. Veränderungen. Zeits. f. Exper. Path. u. Ther., vol. 1, p. 649, 1905.
Drummond, W. B. Histological changes produced by the injection of adrenalin chloride. Jour. Physiol., vol. 31, p. 81, 1904.
Erb, W. Exper. u. histol. Studien über Arterienerkrankung nach Adrenalininjectionen. Arch. f. Exp. Path. u. Pharmakol., vol. 53, p. 173, 1905.
Herter, C. A., and Richards, A. N. Note on the glycosuria following experimental injections of adrenalin. Med. News, vol. 80, p. 201, 1902.
Wolownik, B. Exper. Untersuch. über d. Adrenalin. Arch. f. Path. Anat., vol. 180, p. 225, 1905.
Papadia, G. Arteriosclerosi da adrenalina. Rev. di Pat. Nerv., vol. 11, p. 113, 1906. [Contains bibliography.]
Biland, J. Ueber d. durch Nebennierenpräparate gesetzten Gefäss- und Organveränderungen. Deutsch. Arch. f. Klin. Med., vol. 87, p. 413, 1906.

[b] Houghton, E. M. Pharmacologic assay of preparations of the suprarenal glands. Amer. Jour. Pharm., vol. 73, p. 531, 1901; National Standard Dispensatory, Hare, Caspari, and Rusby, 1905, p. 1732.
Pharmacology of the suprarenal gland and a method of assaying its products. Jour. Amer. Med. Assoc., vol. 38, p. 150, 1902. [A solution of 1–10,000 was used.]
Franz, F. Aus exper. Arbeiten über Adrenalin, seine physiol. Werthbestimmung u. seine Wirkung. Sammelreferat. Med. Klinik, 1907, p. 99.
Battelli, M. F. Quantité d'adrénaline existant dans les capsules surrénales de l'homme. Compt. Rend. Hebd. Soc. de Biol., vol. 54, p. 1205, 1902.

means of the mercury manometer recording on the drum of a kymograph.

Animals preferred.—Dogs, on account of their greater resisting powers and their closer resemblance to man in responding to drugs, are usually preferred for this class of work, although rabbits are very serviceable. Cats are not so sensitive but are preferred by Elliott.

Principal reference literature.—Details regarding the preparation of the animal as to the mechanical carrying out of the experiment can be found in Essentials of Experimental Physiology, by T. G. Brodie, 1898, p. 168; Klein, Burton-Sanderson, and Brunton, Handbook for the Physiological Laboratory; T. Sollmann, Textbook of Pharmacology, 2d ed., 1906, pt. 3; Pembry, Beddard, Edkins, Hill, McLeod, and Pembry, Practical Physiology; E. Cyon, Methodik der physiologischen Experimente und Vivisection; O. Langendorff, Physiologische Graphik; Edmunds and Cushny, Laboratory Guide in Experimental Pharmacology; Hermann, Experimental Pharmacology; H. C. Wood, jr., Description of the Methods of Investigating the Action of Drugs, International Clinic, vol. 4, p. 12, 1902; D'Arsonval, Gabriel, Chaveau, and Marey, Traité de Physique Biologique; R. Tigerstedt, Lehrbuch der Physiologie des Kreislaufes.

Data concerning the anatomy of the dog, the rabbit, and the cat can be found in Ellenberger and Baum, Anatomie des Hundes; W. Krause, Anatomie des Kaninchens; St. George Mivart, The Cat.

The large Handbuch der experimentellen Pathologie und Pharmakologie of Heinz deals with results rather than with details of methods. There are, however, a few points which are not usually mentioned.

Apparatus.—We have been dissatisfied with the ordinary kymograph driven by a spring, on account of the variation in speed and the necessity for frequent winding. In the case of the expensive Hürthle machine this objection does not hold. For this reason the writer has used an electric kymograph—the one described by him in American Medicine, 1904, volume 8, page 405. This kymograph can be easily made in most machine shops, as the wheels are stock cut. The machine requires very little attention and the paper winds regularly and does not sag. The kymograph made by Blix[a] also overcomes many of the usual difficulties. The speed of the kymograph should be kept uniform and should be noted.

The time markers which have given the most satisfactory results are those described by Marvin[b] under the name "Magnet for recording sunshine and rainfall." This by slight modification can be made

[a] Blix, M. Neue Registrirapparate. Arch. f. Gesam. Physiol., vol. 90, p. 405, 1902.

[b] Marvin, C. F. Anemometry. U. S. Dept. Agr., Weather Bureau, Circ. D, Instrument Div., 2d ed., 1900, p. 47.

to write in a vertical position, and it records the seconds in steplike groups so that they can be counted off at a glance.

A very convenient manometer is the ordinary one of glass made into the form of a U. The internal diameter should be as near as possible to 4 mm., as at this width the least error occurs.[a] To the horizontal portion of the tube a small right-angular tube is sealed, and this is connected with the pressure bottle. At the end of the horizontal portion a small flexible lead tube is connected by means of thick-walled rubber tubing, and the opposite end of the lead tube connects with a wash-out cannula.

The most convenient form of wash-out cannula is the one which has been used in Professor Howell's laboratory for ten or fifteen years and consists of a metallic T tube. The long arm of the T is divided in its lower half by a longitudinal partition, which separates this part of the tube into two sections, one continuing on through the full length, the other communicating with the side arm of the T.[b] When this tube is inserted into the glass cannula and connected with the carotid artery a continuous stream of fluid will wash out any clots which may form.

In recording respiration the writer uses, when necessary, the chest tambour connected with an ordinary Marey tambour, writing directly upon the drum of the kymograph. The secret of obtaining good respiratory tracings lies in the use of extremely thin rubber. For this purpose rubber about as thin as tissue paper is best. For accuracy in reading pressures, a running base line is used. In commercial work where it is necessary to save time, the paper is further divided by a series of equidistant lines running parallel to the base line to facilitate reading off measurements. One adjusted to run at the level of normal pressure is especially desirable for rapid work.

As to the respiration apparatus, at present the most suitable one seems to be that of Meyer[c] because of the alternate force and suction pumps, but that of Hoyt,[d] which is merely a force pump, is very serviceable.

Preparation of animals for testing.—The animal should be carefully anæsthetized both for humanitarian reasons and to render it motionless, as any motion on the part of the animal would vitiate the results. If necessary, curare with morphin may be used to secure immobility; then artificial respiration will be required. While the use of chlore-

[a] Schaefer, E. A. Textbook of Physiology, vol. 2, p. 78.

[b] Hermann, L. Exper. Pharmacology, 1883, p. 101.

[c] Meyer, H. Zwei neue Laboratoriumsapparate. Arch. f. Exp. Path. u. Pharmakol., vol. 47, p. 426, 1902.

[d] Hoyt, J. T. Apparatus for artificial respiration. Jour. Physiol., vol. 27, p. 48, 1901.

tone has disadvantages, it is generally used for this class of work in dogs where the animal is not allowed to recover (0.2 gram per kilo is usually given in alcoholic solution after the hypodermic injection of morphin, or chloretone may be used alone). This does away with the use of a volatile anæsthetic.[a] After the use of large doses of chloretone alone the writer noted that while the animal may be merely drowsy after one hour, a few whiffs of ether will secure good anæsthesia, which will continue. Sometimes as anæsthesia sets in the animal ceases to breathe, but a few strokes of the respiration machine will soon restore the breathing, which will then continue of itself. In the case of rabbits, 3 grams of urethane may be given in solution by mouth, or 1.50 grams subcutaneously,[b] while cats should receive 1.25 grams per kilo. With this anæsthetic and kept on a warm table, the blood pressure in cats will remain at 130–140 mm. for hours.[c] Urethane is not suitable for dogs. Before connecting the animal to the kymograph it should be weighed, so that the necessary dose of adrenalin can be calculated.

Results obtained by various investigators.—Cameron[d] has shown that the smallest dose which gives a "definite and invariable rise" in blood pressure in rabbits weighing about 2,000 grams is 0.00062 mg., or 0.0003 mg. per kilo, or 0.00000031 gram per kilogram; that is, 0.5 c. c. of a 0.125 per cent solution of an adrenalin solution of 1 to 1,000, or 0.5 c. c. of a solution of 1 to 800,000 (1 c. c. of a 1–1,000 solution diluted to 800 c. c.).

In cats, which are more resistant than rabbits, Ehrmann[e] found that the intravenous injection of 0.1 mg. gave a rise which was just appreciable.

Abel's epinephrin bisulphate in 0.00013 gram caused a rise of 14 mm. Hg in a small dog with cut vagi,[f] and von Fürth's suprarenin iron compound in doses of 0.000017 gram per kilo (dog) caused the maximum rise, while 0.000075 gram caused a marked rise, 24 mm. Hg in rabbits (2 kilos).[g]

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[a] Impens, E. Chlorétone. Arch. Internat. de Pharmacodynamie, vol. 8, p. 77, 1901. Houghton, E. M., and Aldrich, T. B. Chloretone. Jour. Amer. Med. Assoc., vol. 33, p. 777, 1899.

[b] Schmiedeberg, O. Ueber d. pharmakol. Wirkungen u. d. therap. Anwend. einiger Carbaminsaure-ester. Arch. f. Exper. Path., vol. 20, p. 203, 1886.

[c] Elliott, T. R. Action of adrenalin. Jour. Physiol., vol. 32, p. 449, 1905.

[d] Cameron, I. D. On the methods of standardising suprarenal preparations. Proc. Roy. Soc. Edinburgh, vol. 26, p. 161, 1905.

[e] Ehrmann, R. Ueber eine physiol. Werthbestimmung des Adrenalins. Arch. f. Exp. Path. u. Pharmakol., vol. 53, p. 106, 1905. [Weight of animal not given.]

[f] Abel, J. J. Ueber d. blutdruckerregenden Bestandtheil der Nebenniere, das Epinephrin. Zeits. f. Physiol. Chem., vol. 28, p. 339, 1899.

[g] Von Fürth, O. Zur Kenntniss d. brenzkatechinähnlich. Substanz d. Nebennieren. Zeits. f. Physiol. Chem., vol. 29, pp. 115 and 112, 1900.

Epinephrin sulphate intravenously injected into atropinized dogs previously narcotized with morphin and ether produced a rise as follows:

0.083 millionth of a gram per kilo body weight, 5 mm. Hg.
0.23 millionth of a gram per kilo body weight, 7 mm. Hg.
0.49 millionth of a gram per kilo body weight, 15 mm. Hg.
0.69 millionth of a gram per kilo body weight, 20 mm. Hg.
1.7 millionth of a gram per kilo body weight, 24 mm. Hg.
5.7 millionth of a gram per kilo body weight, 66 mm. Hg.

Unfortunately, in these experiments the interval of time between the injections is not specified.[a]

In a dog weighing 4.5 kilos with a carotid pressure of 140 mm. Hg the intravenous injection of 0.02 mg. of adrenalin was followed by a rise of 46 mm. After the injection of atropin the same dose caused a rise of 50 mm.[b]

The normal blood pressure in rabbits as measured from the carotid artery is about 90 mm. Hg, but varies from 80 to 100.

Josué[c] noted that while frequently repeated intravenous injections caused a permanent rise in blood pressure, yet these animals still gave the characteristic abrupt rise in blood pressure on fresh injections—in other words, there was no immunity—and Elliott and Durham in three experiments with cats failed to find the presence of an anti-body.[d]

The normal blood pressure for dogs, as measured from the carotid artery, is 140 mm. and in rabbits 70 mm. of mercury,[e] while the maximum pressure according to Elliott is ±300 mm. for dogs, 180 mm. for rabbits, and 240 mm. for cats.

Oliver and Schaefer[f] have claimed that 0.015 gram per kilo of the fresh glands would cause the maximum rise in dogs (10 kilos).

In non-narcotized and non-curarized rabbits Pruszynski[g] noted a rise of 90 mm. Hg with 0.000047 gram per kilo of adrenalin. With

[a] Hunt, R. On the effects of intravenous injections of minimal doses of epinephrin sulphate upon the arterial blood pressure. Proc. Amer. Physiol. Soc., p. vii; Amer. Jour. Physiol., vol. 5, 1901.

[b] Baylac, J. Recherches expér. sur les propriétés physiol. et toxiques de l'adrénaline. Arch. Med. de Toulouse, vol. 12, p. 265, 1905.

[c] Josué, O. La pression artérielle chez le lapin à la suites d'injections répétées d'adrénaline dans les veines. Comp. Rend. Hebd. Soc. de Biol., vol. 59, pt. 2, p. 319, 1905.

[d] Elliott, T. R., and Durham, H. E. On subcutaneous injections of adrenalin. Jour. Physiol., vol. 34, p. 490, 1906.

[e] Langendorff, O. Physiolog. Graphik., p. 204.

[f] Oliver, G., and Schaefer, E. A. Physiological effects of extracts of suprarenal capsules. Jour. Physiol., vol. 18, p. 235, 1895.

[g] Pruszynski, J. Influence of adrenalin on the circulatory system. Medicine, vol. 11, p. 924, 1905.

an intravenous injection of 1 c. c. of a 1–100,000 solution, Takamine obtained a rise of 30 mm. Hg in the case of a dog weighing 8 kilos, while in a dog weighing 15.5 kilos 0.000016 gram induced a rise of 9 mm.[a]

With a similar injection of 1 c. c. of a 1–10,000 solution, John[b] obtained a rise of 50 mm., registered by a Hürthle manometer, in a dog weighing 3½ kilos when the vagi were not cut. These results remained constant on repeating. If one vagus nerve was cut the pressure rose in one case 75 mm., and on repeating rose 60 mm. If both nerves were cut the pressure rose much higher—about 150 mm.

According to Toujan[c] 0.001 mg. of adrenalin will cause an appreciable rise in atropinized dogs weighing 10 kilos, a sensibility of 1–1,000,000, and Sollmann reports a rise of 14 mm. after the injection of 0.001 mg. per kilo. Carnot and Josserand obtained a maximum rise of 17.5 cm. Hg in a dog weighing 15 kilos with 0.000016 gram per kilo of adrenalin.[d]

A dog weighing 11.29 kilos anæsthetized with 0.3 gram of morphin intravenously after an injection into the vein of 0.001 gram of adrenalin gave a rise from 38 mm. normal to 128 mm. Hg. A second injection gave a rise from 36 mm. to 132 mm.[e] In Dupuis and Van den Eeckhart's hands an intravenuos injection of 1 c. c. of a 1–4,000 solution of adrenalin increased the blood pressure in the femoral artery 4 cm. Hg in a dog weighing 23 kilos, under morphin and atropin. This rise persisted 1½ minutes. One c. c. of a 1–1,000 solution produced a rise of 14 cm. This returned to normal in five minutes. The rise was followed by a hypotension of 1 cm. below normal.[f]

Sudden death may at times occur in dogs even from 0.12 mg. of adrenalin, although the rise in blood pressure may have been only small (32 mm.). In these cases the action seems to fall directly on the heart, as the respiration may continue after the heart stops.[g]

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[a] Takamine, J. Adrenalin, the active principle of the suprarenal glands. Amer. Jour. Pharm., vol. 73, p. 530, 1901; Therap. Gaz., vol. 25, p. 223, 1901.

[b] John, K. Nebennierenpräparate. Dissert., Leipzig, 1906, p. 17.

[c] Toujan, G. Recherches expér. sur l'adrénaline. Thèse, Toulouse, 1905, p. 36.

[d] Carnot, P., and Josserand, P. Les différences d'action de l'adrénaline sur la pression sanguine suivant les voies de pénétration. Comp. Rend. Hebd. Soc. de Biol., vol. 54, p. 1473, 1902.

[e] Reichert, E. T. Adrenalin, the active principle of adrenal extract. Univ. Pa. Med. Bul., vol. 14, p. 53, 1901.

[f] Dupuis and Van den Eeckhart. L'adrénaline. Ann. de Méd. Vét., vol. 52, p. 484, 1903.

NOTE.—Other figures may be found in Neujean, V., Contrib. à l'étude expér. de l'adrénaline, Arch. Internat. de Pharmacodynamie, vol. 13, p. 45, 1904.

[g] Elliott, T. R. Action of adrenalin. Jour. Physiol., vol. 32, p. 465, 1905.

Livon, C. Danger du principe actif des capsules surrénales dialysé. Comp. Rend. Hebd. Soc. de Biol., vol. 54, p. 1501, 1902.

In cats under urethane, 0.03 mg. of adrenalin produced the maximum rise.[a]

Subcutaneous injections[b] or administration per os usually produced no rise in blood pressure. The slight rise seen at times may be due to the local action on the stomach walls. Intramuscular injections are, however, followed by some rise,[c] and the effects vary with the mode of injection as to whether it passes through the hepatic circulation or through the muscles, etc.[d]

If the injection of small doses is rapidly repeated the rise in blood pressure with the same amount of active principle is slightly less but more persistent.[e] With medium doses the rise and its duration may be greater than from the first, but if a proper interval of time elapses between the injections the second rise will correspond to the first, an observation which is confirmed by Baylac.[f] For a dog of 8 kilos, five minutes' time is sufficient when using small doses—0.006 to 0.001 mg.[g] Josserand says there is no accumulation with 0.000016 mg. per kilo.[h]

If large doses are used, the pressure may stay high for a long time, then fall below normal,[i] and serious disturbances with the heart, shown by pericardial effusions, may occur and vitiate the result.[j] Weiss and Harris[k] have claimed that adrenalin can still be found in the blood even when the blood pressure has fallen to normal; thus after injecting 0.0034 gram of adrenalin into a cat weighing 3,000 grams and waiting thirty minutes, the transfused blood caused a rise of 15 mm. in a second cat. No control experiments with normal blood seem to have been made. Elliott, however, says that when the blood pressure

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[a] Elliott, T. R. Action of adrenalin. Jour. Physiol., vol. 32, p. 448, 1905.

[b] Reichert, E. T. Adrenalin. Univ. Pa. Med. Bul., vol. 14, p. 51, 1901.

[c] Meltzer, S. J., and Auer, J. Ueber d. Resorption aus den Muskeln. Zent. f. Physiol., vol. 18, p. 689, 1904.

[d] Carnot, P., and Josserand, P. Des différences d'action de l'adrénaline sur la pression sanguine suivant les voies de pénétration. Comp. Rend. Hebd. Soc. de Biol., vol. 54, p. 1472, 1902.

[e] Toujan, G. Recherches expér. sur l'adrénaline. Thèse, Toulouse, 1905, p. 37.

[f] Baylac, J. Recherches expér. sur le propriétés physiol. et toxique de l'adrénaline. Arch. Méd. de Toulouse, vol. 12, p. 252, 1905. [Used 10-minute interval for rabbits.]

[g] Toujan, G. Recherches expér. sur l'adrénaline. Thèse, Toulouse, 1905, p. 38.

[h] Josserand, P. Contrib. à l'étude physiol. de l'adrénaline. Thèse, Paris, 1904, p. 33.
NOTE.—See also Gioffredi, C., La distruezione dell' adrenaline nell' organismo, Archiv. di Farmacol. Speriment., vol. 6, pp. 127, 145, 1907.

[i] Battelli, F. Transformation de l'adrénaline dans l'organisme. Compt. Rend. Hebd. Soc. de Biol., vol. 54, p. 1518, 1902.

[j] Elliott, T. R. Action of adrenalin. Jour. Physiol., vol. 32, p. 444, 1905.

[k] Weiss, O., and Harris, J. Zerstörung des Adrenalins im lebenden Tier. Arch. f. Gesam. Physiol., vol. 103, p. 510, 1904.
Harris, J. Dissert., Königsberg, 1904.
Battelli, F. Transformation de l'adrénaline dans l'organisme. Compt. Rend. Hebd. Soc. de Biol., vol. 54, p. 1519, 1902.

has returned to normal in the cat. the substance has then disappeared almost entirely from the blood. Elliott's table showing the effects of injecting smaller doses gives the different results obtained:

CAT (VAGI CUT).

Time.	Treatment.	Results.
11.45.	Received 0.18 mg. adrenalin.	Blood pressure rose 150 to 220 mm. Hg.
11.48.	Received 0.30 mg. adrenalin.	Blood pressure rose 130 to 230 mm. Hg.
11.56.		Blood contained no demonstrable adrenalin.
12.05.	Received 0.3 mg. adrenalin.	Blood pressure rose 145 to 220 mm. Hg.
12.08.	Received 0.6 mg. adrenalin.	Blood pressure rose 110 to 210 mm. Hg.
12.14.	Received 0.6 mg. adrenalin.	Blood pressure rose 130 to 220 mm. Hg.
12.22.	Received 0.6 mg. adrenalin.	Blood pressure rose 120 to 206 mm. Hg.
12.26.		Blood contained no adrenalin.
12.40.	Received 1 mg. adrenalin.	Blood pressure rose 110 to 200 mm. Hg.
12.46.	Received 1 mg. adrenalin.	Blood pressure rose 140 to 178 mm. Hg.
12.50.		Pupils still dilated. Blood pressure 150 mm. Hg.
1.17.	Received 1 mg. adrenalin.	Blood pressure rose 95 to 170 mm. Hg.
1.21.	Received 1 mg. adrenalin.	Blood pressure rose 130 to 156 mm. Hg.
1.24.	Received 1 mg. adrenalin.	Blood pressure 140; no rise.
1.25.		Adrenalin found in blood. Five c. c. contained 0.02 mg.
1.45.	Received 1 mg. adrenalin.	Blood pressure rose 60 to 115 mm. Hg.
1.48.		No demonstrable adrenalin.

Control cat showed 0.01 mg. in 5 c. c.

In comparing the strengths of solutions it should be remembered that because 1 c. c. of a solution gave a rise of 17 mm. Hg, 2 c. c. will not necessarily double this rise; in fact, while there is a general increase with increasing doses up to a certain limit, we are unable to find any mathematical relationship between the rise in blood pressure and the dose. Thus Toujan in a dog narcotized with chloralose and atropinized (1cgm. per 7 kilos), injected 1 c. c. of a suprarenal extract containing 0.001 mg. per cubic centimeter and obtained a rise of 17 mm. After five-minute intervals—

Injected 2 c. c. and obtained a rise of 19.5 mm.
Injected 3 c. c. and obtained a rise of 25.0 mm.
Injected 4 c. c. and obtained a rise of 23.0 mm.
Injected 5 c. c. and obtained a rise of 25.0 mm.

The persistency of the rise gives a better idea as to the intensity of the action: thus, using a drum which traveled at a rate of 0.28 cm. per second, after injecting 1 c. c. of the solution specified the drum traveled 30 mm. before the blood pressure returned to its original level—

After 2 c. c. it traveled 40 mm.
After 3 c. c. it traveled 102 mm.
After 4 c. c. it traveled 130 mm.
After 5 c. c. it traveled 150 mm.

Young dogs are claimed to be more responsive to adrenalin than older dogs having the same weight.[a]

The quantitative work which has been carried on with adrenalin has been performed with the adrenalin of commerce. This was shown to contain extraneous matter (phosphates)[b], as one would expect from the method of isolation described by Takamine, so that it does not seem rational to standardize against a body which is not chemically pure and which the manufacturers themselves do not label as c. p. The standard used should be the highest grade of active principle known. Abel's latest method, as acknowledged by Pauly[c], or adrenalin as prepared by Aldrich's method seems to answer this demand. This adrenalin (Aldrich's) can perhaps be obtained from the manufacturers, but at a higher price. A supply of this high grade adrenalin (epinephrin) should be kept on hand in a vacuum desiccator and preserved in the dark. When the test is to be made, a few milligrams of this are accurately weighed off and dissolved in water with a little over the calculated amount of HCl $\frac{N}{10}$, constantly shaking,[d] and made up in a proportion of 1 to 50,000, the solution placed in a Florence flask and gently heated on the bath to body temperature (37½° C.). One-half of a cubic centimeter of this solution would correspond to 0.001 mg. per kilo for a dog of 10 kilos. One-half of a cubic centimeter of this warm solution is then

[a] Josserand, P. Contrib. à l'étude physiol. de l'adrénaline. Thèse. Paris, 1904, p. 31.

[b] Abel, J. J. On a simple method of preparing epinephrin and its compounds. Bul. Johns Hopkins Hospital, vol. 13, p. 30, 1902. Prof. Abel says: "I judge from the bulkiness of the phosphomolybdate precipitate that a quantitative experiment would result in a high percentage of impurity. * * * Commercial adrenalin was * * * purified * * * and more than thirty analyses of various fractions have been made, but it has been found impossible to secure uniformity of composition among the various products."

Function of the suprarenal glands. Contributions to Med. Research, dedicated to V. C. Vaughan, p. 155.

Compare also Gunn, A., and Harrison, E. F. A new characteristic reaction of adrenaline. Pharm. Jour., 1907, p. 718.

[c] Abderhalden, E., and Bergell, P. Münch. Med. Woch., vol. 51, p. 1003, 1904.

[d] Takamine says "100 parts of adrenalin needs nearly 19 parts of hydrochloric acid in forming a neutral salt." (Amer. Jour. Pharm., vol. 73, p. 527, 1901.)

injected by means of a small calibrated syringe and connections into the saphenous vein. The time of this injection should be registered and should occupy about five seconds, and should be gently performed. This is then rapidly followed from the same syringe with 1 c. c. of the normal salt solution to wash into the circulation any of the solution that may be in the syringe and connections. This injection should occupy the same length of time and should always be noted. It is important that the standard solution be made fresh at each test, as otherwise it gradually loses its activity. The pupils should be examined to see if there is any action, as there may be bodies present in the extract which might neutralize the blood-pressure-raising action. During the experiment the animal must be kept warm. Not over 2 c. c. of fluid should be injected at any one time, to avoid the pressor action of the large volume of fluid. The action of the comparatively large amounts of fluid used in this form of injection may be overcome by injecting the fluid into the vein without the use of a syringe. For this injection a pipette of 1 c. c. capacity accurately divided into tenth cubic centimeters is closely connected to a fine, clean, sharp hypodermic needle by means of a rubber tube, and after gently pushing the needle through the wall of the vein toward the heart the fluid is let run into the circulation and the amount controlled by the finger or, better, a burette may be connected with the cannula and the injection thus made. By this method it will not be necessary to use sodium chlorid solution to wash the adrenalin solution into the vein.

The most satisfactory method is to connect the syringe or, preferably, a burette containing the standard solution with one saphenous vein and the solution to be tested with the corresponding vein of the other leg, so that alternate injections of standard and unknown solution can be made under the same conditions. The writer's own preference is for the burette method.

The maximum rise in blood pressure from this injection, 0.001 mg. per kilo, which is about 14 to 28 mm. Hg, although this will vary in various dogs, is now noted, and also the distance traveled by the paper and the time which elapses before the blood pressure returns to normal. A line running on a level with the base of the blood pressure curve will aid in rapid calculation. The method of measuring the blood pressure can be found in W. Stirling's Outlines of Practical Physiology, third edition, 1898, page 305.

Very dilute solutions are purposely used, so that there will be no accumulation of the active principle in the animal. After five or six minutes a very dilute solution, one which would approximate in strength that of the normal, and diluted to the same bulk as the control, is similarly injected, with all the above precautions, and the

maximum blood pressure and the time which elapses before the blood pressure returns to normal is likewise noted and compared with the standard. At intervals of five or six minutes various amounts of this fluid are injected, until an amount is injected which gives a rise which corresponds to the 1 c. c. of the standard solution. This injection should be repeated several times, changing the order of the injection, and a mean taken, and finally the correct solution and the standard one injected into a fresh dog which has had no fluid injected. By comparing the strength of the two solutions the actual number of milligrams present can be determined. Thus, if in 1 c. c. of the standard solution there were 0.01 mg. and this gave a rise of 14 mm. of Hg, and if 2 c. c. of a similar dilute solution of the unknown gave the same rise, we would naturally argue that the second solution was one-half the strength of the first and contained 0.005 mg. per c. c. The solutions of the unknown can then be adjusted, so as to use the same amount of fluid of the standard and the test controlled, reversing the order of the injections. It is advisable to repeat the injections several times, checking these results repeatedly.

Elliott[a] advises against using over 0.03 mg. Dale, using cats according to Elliott's method, is said to measure epinephrin solutions within about 5 per cent.[b]

Cameron,[c] struck with the work of Marshall on the antagonism of the members of the digitalis series with the nitrites, extended these observations to the antagonism between the nitrites and adrenalin and found that 0.6 mg. ($\frac{1}{100}$ gr.) of nitroglycerin would require 0.0075 mg. of adrenalin to neutralize its vasodilator action in a rabbit weighing 2,000 grams anæsthetized with ether. This method he considers even more satisfactory than the simple blood pressure measuring, especially if there is not a reliable standard preparation to standardize against. One example from Cameron will illustrate. The minimal effective dose of a 1 per cent solution was 0.5 c. c. The minimal effective dose of an adrenalin solution had been found to be 0.00062 mg. for rabbits of 2,000 grams weight; hence, 0.005 c. c. of the solution = 0.00062 mg. adrenalin, or 1 c. c. = 0.12 mg. By the nitrite method 0.6 mg. is neutralized by 0.7 c. c. of 10 per cent; this amount of nitroglycerin is neutralized by 0.5 c. c. of 1.5 per cent adrenalin solution (0.0075 mg.); therefore, 1 c. c. = 0.107 mg. This solution was one-tenth stronger than adrenalin chlorid, 1–1,000.

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[a] Elliott, T. R. Action of adrenalin. Jour. Physiol., vol. 32, p. 448, 1905.

[b] Barger, G., and Ewins, A. J. Note on the molecular weight of epinephrin. Chem. News, vol. 93, p. 90, 1906.

[c] Cameron, I. D. On the methods of standardising suprarenal preparations. Proc. Roy. Soc. Edinburgh, vol. 26, p. 170, 1906.

Ott and Harris [a] had tried this antagonistic action of these two bodies, but did not push the matter as far as Cameron. A similar antagonism with cholin had been pointed out by Lohmann.[b] After having determined the amount of active principle on the dog, rabbit, or cat by the simple blood pressure method, as a check to this method it may be advisable to inject the amount of the unknown solution corresponding to 0.0075 mg. adrenalin mixed with a solution of 0.6 mg. nitroglycerin into the jugular vein of a rabbit. I do not consider this additional test necessary, but the results of Cameron are certainly worth controlling.

Gürber [c] pointed out the presence of a depressor body in the suprarenal extracts. A portion of this action may be due to cholin and a portion to unknown bodies.[d] Extracts containing these depressor bodies are especially toxic. In crude extracts the depressor action of these bodies, and also of certain inorganic salts, should be kept in mind.

At the end of the experiment the animal should be killed by injecting chloroform into the veins, so that the most humane person could find no objection on the score of cruelty.

In the case of a mixture of adrenalin and peptone the action of one follows that of the other, and it is therefore unsuited for antagonistic study.[e]

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[a] Ott, I., and Harris, S. B. Physiological action of adrenalin chloride. Therap. Gaz., vol. 27, p. 378, 1903.

[b] Lohmann, A. Cholin, die den Blutdruck erniedrigende Substanz der Nebenniere. Arch. f. Gesam. Physiol., vol. 108, p. 222, 1907.

[c] Metzger, L. Zur Kenntniss d. wirksam. Substanzen d. Nebenniere. Dissert., Würzburg, 1897, p. 17.

[d] Hunt, R. Further observations on the blood pressure lowering bodies in extracts of the suprarenal glands. Proc. Amer. Physiol. Soc., Amer. Jour. Physiol., vol. 5, p. vi, 1901.

Marino-Zuco, F., and Dutto, U. Chem. Untersuch. ü. die addison'sche Krankheit. Untersuch. z. Naturlehre. Herausg. von J. Moleschott, vol. 14, p. 617, 1892.

Lohmann, A. Cholin, die den Blutdruck erniedrigende Substanz der Nebenniere. Arch. f. Gesam. Physiol., vol. 108, p. 215, 1907.

Pari, G. A. Azione locale dell' adrenalina sulle pareti dei vasi, ed azione delle minime dose di adrenalina sulla pressione del sangue. Arch. di Farmacol. Speriment., vol. 4, p. 175, 1905.

Vincent, S. Nature of the physiologically active substances in extracts of nervous tissues and blood, with some remarks on the methods of testing for cholin. Jour. Physiol., vol. 30, p. 143, 1904.

[e] Hamburger, W. W. Action of intravenous injections of glandular extracts and other substances upon the blood pressure. Amer. Jour. Physiol., vol. 11, p. 282, 1904.

In the report made to the Council on Pharmacy and Chemistry of the American Medical Association, no attempt was made to check up the results with the rise obtained from a definite amount of pure adrenalin, but only to compare the activity of the preparation on the market. Sollmann and Brown wisely remark that their results give no idea as to the activity of the preparations before leaving the manufacturers' hands.[a]

An interesting commercial problem is to see by what means the yield of adrenalin can be increased. Toujan has claimed that in the products of auto-digestion of pancreas there is a body (not tyrosin) which added to the ground-up suprarenals does this,[b] while Halle has found that the addition of tyrosin to the ground-up suprarenals caused in some cases an increase in the adrenalin yield.[c] Others claim that the addition of ground-up muscle added to suprarenal extract does the same.[d]

TOXICITY OF THE ACTIVE PRINCIPLE.

The following data on the toxicity of adrenalin may be of use:

The lethal dose for frogs is over 0.50 mg. per kilo. The relatively large amount necessary to kill is due to the fact that pulmonary respiration in frogs is not indispensable. In animals death is usually due to pulmonary œdema and an action on the heart.

The subcutaneous injection of 0.01 gram per kilo kills guinea pigs, but 0.004 gram rarely.

In rabbits 0.02 gram per kilo given hypodermically is fatal.[e] Baylac gives the immediate toxicity by this method as 1 cg. per kilo for rabbits and guinea pigs. One milligram adrenalin subcutaneously injected in rabbits weighing 400 grams rapidly kills.[f] On the intravenous injection into rabbits, 0.0006 gram per kilo is always fatal,[g] while three out of four die from 0.0004 gram per kilo. The imme-

[a] Sollmann, T., and Brown, E. D. Comparative physiologic activity of some commercial suprarenal preparations. Jour. Amer. Med. Assoc., vol. 47, p. 792, 1906.
NOTE.—Compare also Hunt, R., Comparative physiologic activity of some commercial suprarenal preparations, in Jour. Amer. Med. Assoc., vol. 47, p. 790, 1906.

[b] Toujan, G. Recherches expér. sur l'adrénaline. Thèse, Toulouse, 1905, p. 67.

[c] Halle, W. L. Ueber d. Bildung d. Adrenalins im Organismus. Beitr. z. Chem. Physiol. u. Path., vol. 8, p. 276, 1906.

[d] Abelous, G. E., Soulié, A., and Toujan, G. Influence des extraits des organes et des tissus animaux soumis a l'autolyse sur la production de l'adrénaline. Comp. Rend. Hebd. Soc. de Biol., vol. 60, p. 16, 1906.

[e] Taramasio, P. Étude toxicol. de l'adrénaline. Thèse, Genève, 1902, p. 30.

[f] Paton, D. N. Effect of adrenalin on sugar and nitrogen excretion in the urine of birds. Jour. Physiol., vol. 32, p. 62, 1905.

[g] Battelli, F. Toxicite de l'adrénaline en injections intraveineuses. Comp. Rend. Hebd. Soc. de Biol., vol. 54, p. 1247, 1902.

diate toxicity by vein in rabbits (2 kilos) is 0.06 milligrams per kilo.[a] In dogs from 0.1 to 0.2 mg. per kilo intravenously injected kills, while in cats from 0.5 to 0.8 are sufficient.[b] Twenty milligrams adrenalin subcutaneously injected in cats caused no disturbance until the following day (Elliott).

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[a] Baylac, J. Recherches expér. sur les proprietes physiol. et toxiques de l'adrénaline. Arch. Méd. de Toulouse, vol. 12, p. 247, 1905.

[b] Lesage, J. Recherches expér. sur l'adrénaline. Arch. Internat. de Pharmacodyn., vol. 13, p. 273, 1904.

NOTE.—See also Amberg, S., Toxicity of epinephrin, in Proc. Amer. Physiol. Soc., p. xxxiii, Amer. Jour. Physiol., vol. 8, 1903.

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