FACTORS AFFECTING EPINEPHRINE CONTENT OF ADRENAL GLANDS

C. G. VAN ARMAN

From the Department of Pharmacology, Northwestern University Medical School
CHICAGO, ILLINOIS

MUCH of the work in the literature concerning the epinephrine content of the adrenal glands can be criticized for depending upon non-specific assays, for not being statistically significant, or for giving insufficient data for proper evaluation. This paper attempts to avoid these pitfalls and is concerned with 1) relative epinephrine content in left and right adrenals, 2) epinephrine content as a function of adrenal gland weight, body weight and sex, 3) effect of unilateral adrenalectomy, and 4) the role of innervation in synthesis of epinephrine.

METHOD OF ASSAY

No colorimetric method at present is suitable for the purpose at hand. The only chemical method which is specific is the fluorimetric procedure as used by Pekkarinen (1), but this does not have sufficient accuracy. Chromatography is not sufficiently quantitative.

Biological assay employing the pressor response of the atropinized dog is specific enough for the epinephrine content of adrenal glands. The only other compound having significant pressor effects, which is likely to occur in the adrenals, is nor-epinephrine. The procedure developed by Noel (2) has been adapted to the present studies. Computation of the assay results is by statistical means which give the potency of the unknown solution and its standard error as percentage of the value found. The average standard error of 18 rat-adrenal assays in the current experiments was 3.5 per cent and was constant throughout the range of 15 to 150 gammas of epinephrine in a 10-cc. extract. By using a dog more sensitive than usual, and employing pyribenzamine to increase the responses (5 mg/kg., i.p.), amounts as small as 6 or 8 gammas may be determined, with a somewhat larger standard error. The standard was U.S.P. reference standard epinephrine (control 1949-1 II), which possibly contains some nor-epinephrine (3).

The procedure for making the extract is as follows: The gland is quickly removed from the freshly killed or anesthetized rat, and stripped of adhering fat. It is weighed and ground with a small amount of sand moistened with acid saline (0.85% saline with 1 cc. glacial acetic acid per 100 cc.). Grinding is continued until no particles of gland or sand are visible. Five cc. of acid saline are added and stirred, then decanted into a 30-cc. beaker. The residue is ground a bit more, then 4 cc. more of acid saline
are added, stirred and decanted. The solution is now brought to a boil and filtered through glass wool into a 10-cc. volumetric flask. The mortar and beaker are rinsed with 2 cc. of acid saline, heated, and the 2 cc. are used to rinse the funnel and bring the volume up to 10 cc. The assay is preferably performed at once, but refrigeration overnight has no effect.

A test of the reliability of this extraction technique was made by substituting in place of the adrenal gland a known amount of standard epinephrine. Assays upon 4 ‘extracts’ gave a mean result of 85.5 per cent of the epinephrine added, with a standard deviation of 1.7 per cent. The method is thus reliable, but has a fairly consistent loss of about 15 per cent. No further detectable amount of pressor substance was obtained from the residue after the above extraction.

RESULTS AND DISCUSSION

Relative Epinephrine Content in Left and Right Adrenal Glands. All workers agree that the left and right adrenal glands in dogs, cats and rabbits have equal amounts of epinephrine, within the limits of error of the methods used (4–8). Almost no data concerning the rat have been found in the literature.

Results obtained from 21 normal rats of various strains and ages, of both sexes, using the assay method described above, show that the right adrenal contains 98.5 per cent as much epinephrine as the left, with standard deviation of 13.7 per cent and standard error of 3.0 per cent. There is, therefore, on the average, no significant difference between left and right glands; however, in any individual rat there may be a considerable difference. In most cases the difference cannot be attributed entirely to errors in the assay. A high degree of correlation, 0.94, exists for left and right adrenal contents in this series.

Epinephrine as a Function of Adrenal Gland Weight. Figure 1 shows a good positive correlation between the weight of freshly trimmed adrenal glands and their epi-
nephrine content, in 29 individual rats and in 12 groups of 4 rats each, of various strains and ages, and of both sexes.

Epinephrine Content as a Function of Body Weight and Sex. As figure 2 shows, the epinephrine store of the adrenal gland is related to the body weight. The sex difference appears to be due mainly to the fact that the adrenal glands of the female are larger in proportion to body weight. The data were taken from 25 individual rats and 7 groups of 4 rats each.

The correlation between body weight and adrenal gland weight observed by Hatai (9) was confirmed in the present study, in 70 male and female rats of various strains and ages.

Effect of Removing One Adrenal Gland Upon Epinephrine Content of Remaining Gland. It is well known that removal of one adrenal gland causes a considerable increase in weight of the remaining gland. Hypertrophy of the cortex seems to be generally accepted, but opinions differ regarding hypertrophy of the medulla (6, 7, 10).

Eight mature rats of both sexes and of various strains were individually caged for a few days, then adrenalectomized on the left side. After a number of days, namely 5, 6, 6, 7, 8, 8, and 53, the right adrenal was removed by a similar operation. Assay results show that the right gland contained 98.4 per cent as much epinephrine as the left, on the average, with no correlation in regard to time elapsed between operations.

These results were confirmed in 44 other rats, i.e., 11 groups of 4 rats each, in which the left glands had been first removed, and after a number of days, the right glands. These were female albino rats (Sprague-Dawley) of 300–360 gm. weight. The 4 glands from each group were pooled together for assay. Days elapsed between the two operations were as follows: 16, 19, 25, 25, 27, 27, 28, 28, 30, 30, and 36. Epinephrine content of the right glands was 43.0 ± 1.1 gammas per gland (mean and standard error), again with no correlation with respect to time elapsed. This value is to be compared with the control value 44.6 ± 0.8 gammas per gland found in 5 groups of 4 normal rats each. It is obvious that removal of one adrenal had no effect upon the epinephrine content of the remaining adrenal, in the interval from 5 to 53 days.

A decided increase in weight of the right gland over that of the left was recorded in the great majority of individuals, whereas in normal rats the left gland is slightly
heavier. The accompanying table shows the weight of the remaining (right) adrenal at various intervals after removal of the left, expressed as percentage of the weight of the left gland. These data are taken from the 44 rats mentioned in the preceding paragraph, plus 95 other rats of several strains. Differences between controls and other values are statistically significant at every interval shown.

Hypertrophy of the gland as a whole had thus taken place without any increase in the epinephrine content. This argues for hypertrophy of the cortex alone.

Necessity of Innervation of the Adrenal Gland for the Synthesis of Epinephrine. It has been well established that the splanchnic innervation of the adrenal gland is required for the discharge of epinephrine. One might inquire whether it is necessary also for the synthesis or storage of epinephrine within the gland. Crowden (8) investigated this question in cats, and concluded that the innervation is not required. It seemed that the non-specific calorimetric assay employed by Crowden left room for doubt regarding his conclusion. Possibly the adrenal gland could, in the absence of innervation, accumulate compounds which would give the color reactions similar to those of epinephrine, but could not actually achieve the entire synthesis. In order to clear up

| TABLE 1. WEIGHT OF REMAINING (RIGHT) ADRENAL GLAND AT VARIOUS INTERVALS AFTER REMOVAL OF LEFT GLAND |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| DAYS, NO. OF RATS | WEIGHT OF RIGHT AS PERCENTAGE OF LEFT | DAYS, NO. OF RATS | WEIGHT OF RIGHT AS PERCENTAGE OF LEFT |
| None, 15 | 02 ± 3.2 | 16 to 20, 20 | 124 ± 3.2 |
| 4, 12 | 128 ± 3.2 | 21 to 30, 53 | 111 ± 2.1 |
| 5, 13 | 133 ± 4.7 | 30 to 53, 12 | 134 ± 6.6 |
| 7 to 9, 14 | 121 ± 4.2 | | |

1 Mean and standard error.

this point, therefore, somewhat similar experiments have been performed in rats, using the biological method of assay on dog’s blood pressure.

The equality of epinephrine store in left and right adrenal glands of the rat has already been demonstrated. A similar equality also exists in rats which have been denervated on the left side by the technique of stripping the margo superior of the entire sheet of tissue which extends along it, containing splanchnic fibers and the main arteries. Comparison of epinephrine load in left and right glands 3 days after the operation showed good agreement, in 6 rats (right equalled 102.2% of left).

Seven rats, denervated by the foregoing technique, were given physostigmine intraperitoneally, 2 mg. per kg., and allowed water but no food. After 17 hours the innervated gland was uniformly depleted as compared with the denervated one, the mean depletion being 43 per cent. Recovery of normal glands from this dose of physostigmine was demonstrated after a 3-day recovery period in one group of 4 normal rats (found, 45.2 7/gland; normal value, 44.6).

As the next step, 4 normal female rats (Wistar, approximately 4 months old) were injected with physostigmine. After 17 hours, the left adrenals were quickly denervated under ether anesthesia. The rats were then allowed to recover on a full diet.
for 3 days. At the end of this period, the denervated left glands contained 114, 155, 114, and 104 per cent as much epinephrine as the intact right glands.

The biological method of assay used here eliminates the possibility that the denervated adrenal may have accumulated physiologically inactive catechol-like precursors of epinephrine instead of the hormone itself. It gives no information about the relative amounts of nor-epinephrine before and after denervation; this question should be investigated.

It appears certain that 1) depletion of both glands occurred, 2) the left glands were denervated, 3) the right, i.e. normal, glands had recovered their epinephrine store after 3 days, and 4) the left glands had recovered to an extent at least equal with the right. These results confirm and extend those of Crowden: the adrenal gland can increase its store of epinephrine in the absence of its innervation.

**SUMMARY**

In normal rats the left and right adrenal glands contain equal amounts of epinephrine, on the average; however, the standard deviation of the right expressed as percentage of the left is almost 14 per cent. Data are presented concerning the relationships of adrenal gland weight, body weight and sex to the epinephrine content.

After unilateral adrenalectomy, the epinephrine content of the remaining adrenal is the same as in normal rats, during the period from 5 to 53 days. This finding does not agree with earlier reports based upon non-specific assay methods. The adrenal gland can increase its store of epinephrine in the absence of innervation.

It is a pleasure to acknowledge the able technical assistance of Mr. Howard Bibler.

**REFERENCES**