A NOTE ON THE ABSENCE OF ADRENALIN IN MALIGNANT RENAL HYPERNEPHROMAS.*

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INTRODUCTION.

After calling attention to the lack of observations or of any real effort to determine whether adrenalin or adrenalin-like blood pressure raising substances are present in malignant renal hypernephromas, Greer and Wells (1) report the results of the examination of two specimens, both of which gave entirely negative results. Examination of the literature at my command has not revealed much, besides the work of Greer and Wells, in the way of experimental evidence on this question. In the course of the discussion of a paper, Biedl (2) called attention to the point that all tumors which, when removed by surgical operation or found at autopsy, lead one to suspect by their location or other characteristics that they have developed from medullary adrenal tissue should be examined by making tests for adrenalin in extracts of the tumors; and that when so tested, tumors arising from chromaffin tissue of the medulla should show a specific tonus-heightening and especially vasoconstricting substance, while those tumors arising from the cortical tissue should show no such substance. Davidsohn (3) in discussing a paper refers to the presence of adrenalin in a metastatic tumor of the omentum primary in the adrenal, but no very convincing experimental evidence is given. Negative results were obtained by Taddei (4) from some tests on a malignant hypernephroma removed from a boy eleven years of age. Suzuki (5) described in a man sixty-two years of age a tumor which appeared to consist of both medullary and cortical substances, but he gave no direct experiments on the adrenal content of either portion of the tumor.

From the above it would seem that a chromaffin tumor can arise from medullary tissue, but not from cortical rests, which are the places of origin of true malignant hypernephromas. Such chromaffin tumors might well be expected to contain adrenalin, but thus far such a content has not been demonstrated. On the other hand, the ordinary malignant hypernephroma originating in cortical tissue has been tested and found to contain no adrenalin.

The present note is a continuation of, and is supplementary to the paper by Greer and Wells.

ORIGIN OF SPECIMENS.

One of the specimens (A) was metastatic tissue from an adrenal tumor resembling in many respects a renal hypernephroma, and was

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obtained from an autopsy performed on November 9, 1909, by Dr. H. Gideon Wells, at Cook County Hospital. The pathological report has been published by Dr. B. F. Davis (6). There was found a large primary tumor above the right kidney with metastases scattered through the body, especially in the bones and lymphatics. A mass of the metastatic glands was taken from the peribehatic region and afterwards tested for adrenalin as described below. The other two specimens were typical hypernephromas. One of them (specimen B) was a fine specimen, in good condition, which was removed by surgical operation at the Presbyterian Hospital, by Dr. Dean D. Lewis, about two hours before I received it. The other (specimen C) was also a good specimen and weighed about 300 grams. It was removed from a Presbyterian Hospital patient by Dr. A. D. Bevan, and a portion of it reached me about two hours after removal. Also from one of Dr. L. L. McArthur's patients who had a malignant hypernephroma, a specimen of blood was obtained (specimen D), and was examined for its adrenalin content.

EXPERIMENTAL RESULTS.

Specimen A.—Adrenalin glands which contain adrenalin turn black on exposure to air, or even in the body if it is allowed to lie for some days after death. Variations in the amount of blood present in the glands, however, would greatly alter their color, for blood itself would turn black under the same conditions. Although this patient had died four days previously, neither the primary tumor nor the metastases showed any considerable darkening either at autopsy or when pieces were allowed to stand exposed to the air for several days.

Twenty-five grams of the tumor were ground up with sand and fifty cubic centimeters of 0.9 per cent. sodium chlorid solution, and allowed to stand for two hours; the mixture was then filtered, and the filtrate divided into two parts. One part was tested by its effect on carotid blood pressure when injected into the femoral vein of a dog, and by its effect on the rabbit uterus strip. The other portion was acidulated slightly with acetic acid and boiled, after which it was filtered and tested by the same methods employed for the unboiled portion.
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A small dog was anesthetized with ether and cannulae were inserted by the usual technique; a three-way cannula in the carotid for blood pressure with a mercury manometer, and a cannula in the femoral vein with a burette for the injection of the solution. Repeated injections were made, beginning with small doses (0.5 of a cubic centimeter) and increasing to larger ones (ten cubic centimeters), but no characteristic adrenalin rise in the blood pressure was observed.

When control extracts made from air-dried adrenals, prepared as described above, were used, they gave the adrenalin rise in the blood pressure curve.

Control injections of adrenalin chlorid solution in doses of one to three cubic centimeters of one part in one million, made in 0.9 per cent. sodium chlorid solution, gave a sharp rise in blood pressure showing the usual blood pressure curve.

Rabbit uterus strips (7) were prepared and mounted in Ringer's solution and kept at a temperature of 38° C. with air slowly bubbling through the solution, and allowed to stand until the strips had ceased contracting and had become quiescent. Dilutions of adrenalin chlorid of one part in ten million, one part in five million, and one part in one million, were prepared in Ringer's solution kept at 38° C. with air slowly bubbling through. The irritability of the uterus strips was tested by displacing the pure Ringer's solution containing the different dilutions of adrenalin. If the strips responded well to one part in one million dilution, they were deemed sufficiently irritable to be used for testing; and if they responded well to the higher dilutions they were thought to give a correspondingly more delicate test for the presence of adrenalin. These same strips together with others which were prepared from the same uterus were then used to test the extracts prepared as described above. When this was done it was found that no contraction of the strips was caused by any of the solutions containing various amounts of the hypernephroma extracts.

The extracts prepared as described were allowed to stand in the laboratory at room temperature for twenty-four hours. It was found that no reddish or pinkish coloration occurred in any of the extracts of hypernephroma. But in the extracts of dried adrenal
glands taken from laboratory animals and prepared as already described distinct pinkish coloration occurred. Also similar pinkish coloration always occurred in the solution of adrenalin chloride in sodium chloride solution, or in Ringer’s solution.

Specimen B.—Fifteen grams of the tumor were taken and ground fine with sand in a mortar with thirty cubic centimeters of Ringer’s solution. It was allowed to stand for two hours, and the filtrate injected in various sized doses, under ether anesthesia, into the femoral vein of a small dog which had its carotid artery connected with a mercury manometer. Doses of one cubic centimeter, five cubic centimeters, and ten cubic centimeters were injected without causing any change in the blood pressure except the very slight rise caused by the injection of that amount of fluid. There was no characteristic adrenalin effect obtained, although the trials were repeated until the supply of extract was exhausted.

Extracts of six adrenals, which had been dried in the open air after having been removed from rabbits and dogs previously used in the laboratory, were made by grinding the glands in a mortar with sand and Ringer’s solution, allowing the mixture to stand two hours, and filtering it. When injected intravenously the extracts gave a sharp rise in the blood pressure followed by some fluctuations and irregularities, but in general they showed a very good agreement with the typical adrenalin curve.

Another extract was made with fifteen grams more of the tumor, which were ground in a mortar with fifty cubic centimeters of Ringer’s solution, allowed to stand for two hours, and were then filtered. These extracts were tested with rabbit uterus strips and the results controlled by using solutions of adrenalin chloride to see that the uterus strips were sufficiently irritable to respond to adrenalin in dilution of one part in one million.

Uterus strips were prepared from a large rabbit and two of them were mounted in Ringer’s solution at 38° C. with air slowly bubbling through the solution. After waiting until the strips had become quiescent, a solution of one part in one million of adrenalin in Ringer’s solution at 38° C. was used to displace the pure Ringer’s solution of one of the tubes. The strip in that tube at once contracted strongly. The adrenalin solution was then displaced with
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fresh Ringer’s solution at the same temperature. The strip very soon relaxed again. Then the two strips were used to test the extract of hypernephroma. One cubic centimeter of the extract was poured into the bottom of the tube, which held about twenty-five cubic centimeters, and time was allowed for the extract to be diffused throughout the solution. There was no contraction of the uterus strip. Although the amounts of extracts added were increased to two, five, and ten cubic centimeters until the supply was exhausted, neither strip of uterus showed any contraction.

The remainder of the tumor material was allowed to stand in the open air for several days to see if blackening would occur; but there was no considerable change in color.

Specimen C.—A piece weighing about 150 grams reached me two hours after removal. The technique of the examination of this tumor is not given in detail because it was in all points similar to that described above for the other tumors.

Pieces of this tumor when exposed to the air blackened but slightly, while pieces of normal adrenals which were exposed under the same conditions at the same time blackened very much.

Extracts made as described above, when injected into the femoral vein of a dog, caused no adrenalin-like rise in blood pressure. There was merely a slight temporary upward curve of the blood pressure tracing such as was caused by the injection of so much salt solution. Control extracts made in the same way and at the same time from adrenals from laboratory animals gave the adrenalin rise in the blood pressure curve. Control injections of solutions of adrenalin chlorid showed the usual adrenalin curve.

Extracts of the tumor, when allowed to stand in room temperature, showed no pinkish coloration, while the extracts of adrenal glands of laboratory animals under the same conditions became pinkish in a few hours.

When applied to the rabbit uterus strips, the extracts of the tumor gave no stimulation, while the control extracts of adrenal glands produced strong contractions, and while also the control solutions of adrenalin chlorid, one part in one million, gave powerful contractions.

Specimen D.—The rabbit uterus strip test was applied.
Uterus strips were prepared from a large rabbit and mounted in Ringer's solution with the air slowly bubbling through and with the temperature kept at 38° C. After the preparation had been allowed to stand until the strips had become quiet, dilutions of the blood of the patient varying from one part in one hundred to one part in ten were used to displace the Ringer's solution around the uterus strips. But in no instance was there any contraction of the strips. Controls of adrenalin chlorid solution showed that the strips would respond with a powerful contraction when placed in a solution of one part in one million.

Dilutions of the blood of less than one part in ten were not used because it was thought that if there were a considerable increase above the normal adrenalin content of the blood, a dilution of one to ten ought to give a positive result with the rabbit uterus test; whereas normal blood might give a positive test in the dilution of one to five.

**SUMMARY.**

The results of these experiments show, in short, that, by the method employed, adrenalin could neither be demonstrated in the specimen of blood, nor in either of the two specimens of malignant hypernephroma, nor in the specimen of metastatic tissue from the adrenal tumor which closely resembled a malignant hypernephroma.

The results agree with those of Greer and Wells in showing that no adrenalin is found in malignant renal hypernephromas; and the further observation is made that no adrenalin content of the blood of a patient with malignant renal hypernephroma could be shown by the rabbit uterus strip test.

Those rare tumors which arise from medullary adrenal tissue might be expected to contain adrenalin; but as pointed out by Greer and Wells, since the ordinary malignant renal hypernephroma arises from adrenal rests which consist of cortical tissue, and since the cortex of adrenal contains no adrenalin, no adrenalin should be expected in the tumors arising from such cortical tissue.

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