EFFECTS OF PITUITARY TRANSPLANTATION IN COCKERELS

by

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ABSTRACT

The criteria of pituitary function employed in this study were visible secondary sex characteristics, testis weight, adrenal weight, thyroid structure, and ability of the thyroid to accumulate I^{131}. On the basis of these criteria, four categories of cockerels were compared: intact birds, hypophysectomized birds with pituitaries autotransplanted to the anterior chambers of their eyes, hypophysectomized birds without pituitary transplants, and partially hypophysectomized birds.

Severe atrophy of the pituitary transplants precluded conclusive assessment of the effects of transplantation. With only one clear exception, the hypophysectomized birds with pituitary transplants were not discernibly different from the hypophysectomized birds without transplants. Evidence from the exception suggested that pituitary tissue transplanted to the eye might be capable of maintaining normal thyroid and adrenal characteristics if enough of the transplanted tissue survived. The partially hypophysectomized birds all exhibited the signs of sexual development, but in the expression of the other effects of pituitary function, they were erratic.

INTRODUCTION

Hypophysectomy has been performed on chickens since the turn of the century (Fichera 1905) in the investigation of pituitary function, and a firm consensus on the principal effects of hypophysectomy has appeared in the literature (Mitchell 1929, Hill and Parkes 1934, Nalbandov and Card 1943). Several of these well-documented effects and their antitheses have been used as indices of pituitary function in this study.

Recently, the influence of the hypothalamus upon pituitary function has claimed much attention (Harris 1955), and the disconnection of the pituitary from the hypothalamo-hypophyseal blood vessels has been recognized as an indispensable method of pituitary study. Most commonly, this disconnection has been accomplished and maintained by transplantation of the pituitary to the kidney in chickens (Ma 1963; Rosenberg, Dimick, and La Roche 1963).

The burden of the present study has been to reexamine firsthand several of the effects of hypophysectomy and to use these effects for assessment of the efficacy of the pituitary after autotransplantation into the anterior chamber of the eye.

MATERIALS AND METHODS

Three-month-old White Leghorn cockerels from one flock and two-month-old cockerels from a second flock were hypophysectomized with the essential apparatus and techniques described in detail by Rothchild (1948). The principal modification of the Rothchild method for this study was approach to the drilling site through an incision in the floor of the mouth rather than through the open mouth. Mitchell (1929), Hill and Parkes (1934), and Opel (1965) have described this transbuccal approach to the pituitary and have also supplied much helpful anatomical description of the operating area. The transbuccal approach, though more traumatic to the bird than Rothchild's oral approach, offers the advantage of better visibility of the field of operation.

The birds were anesthetized with sodium pentobarbital, administered usually in a superficial vein at the medial side of the ankle in younger birds and usually in one of the superficial veins at the underside of the wing in older birds. It is easier to insert a needle into the ankle vein without producing hematomas because this vein is less mobile and less fragile than the veins at the undersurface of the wing. However, in older birds this vein is often difficult to find. Anesthetic dosage was determined for each bird by observation of its response to slow injection. The amount given was roughly 0.5 cc per kg of body weight.

The removal of the pituitary was performed under a dissecting microscope, though Rothchild apparently had not deemed the microscope necessary. If the entire anterior pituitary is to be removed, the drill hole must be made as large as possible without damage to the internal carotid artery on each side and the intercarotid anastomosis, which lies across the ventral aspect of the caudal lobe of the anterior pituitary. It is difficult to understand how such a delicate operation could be performed without magnification.

The extirpated pituitary was caught in a gauze trap held in place in the suction cannula with a thread passing through the suction-control aperture and tied around the outside of the cannula. As soon as possible, the pituitary was removed from the trap and placed into chicken Ringer's solution. The bird was taken off the operating board and put into deeper anesthesia with ether. A small incision was cut in the cornea, and the pituitary was inserted through this with a fine forceps.

Immediately after surgery each bird received an injection of 100,000 U.S.P. units of penicillin and five

U.S.P. units of corticotropin (Armour Acthar). The corticotropin injection was repeated once daily until the bird resumed eating and recovered from the usual postoperative droopiness. For some birds two corticotropin injections were deemed sufficient; others continued to receive the injections for as long as five days. Birds that did not receive the corticotropin almost invariably died within three days after surgery. Hypophysectomized birds from which Hill and Parkes (1934) had withheld such temporary substitution therapy had also died soon after surgery. Five of the three-month-old cockerels hypophysectomized for the present study survived through the duration of the experiments of the study, and 12 of the two-month-old birds survived long enough to be useful in the experiments.

Birds from both groups were given intraperitoneal injections of NaI^{131} at the same time. For the older birds this was three months after hypophysectomy and for the younger birds two months after surgery. The dose was 0.5 ml of solution with radioactivity of 0.05 mc. At 5, 10, 25, 50, 75, and 100 hours after injection, the radioactivity of the thyroids was measured with a deep-well scintillation counter. At each measurement the bird was held over the counter in a horizontal position with the convergence of the clavicles on the rim of the well. The number of radioactivity counts per minute was noted, and the whole procedure, including the positioning of the bird, was repeated. The higher of the two readings thus obtained was taken as the measure of the radioactivity of the thyroids. This reading was corrected for radioactive decay by division of the raw count by the percentage of the original I^{131} radioactivity remaining at the time the count was taken. The percentage of radioactivity remaining at a given time was determined with a nomograph constructed on one-cycle semilogarithmic graph paper. Time was plotted along the linear scale of the X axis, and percentage of radioactivity remaining was represented on the logarithmic scale of the Y axis. A straight line drawn from the point representing 100% activity at 0 time to the point representing 50% activity at the half life of the isotope included all the points representing percentages of radioactivity remaining at all intermediate times. The percentage of radioactivity remaining at the time of any count was read directly from the nomograph, and this figure was then used for correction of the raw count taken at that time. The corrected counts obtained in this experiment are recorded graphically in Figs. 1 and 2.

The experiment was repeated two months later for both groups of birds. The radioactivity measurements obtained in this second experiment are recorded in Figs. 3 and 4.

The populations of the two groups of birds were changed slightly between the two radioactivity experiments. Two birds were discarded from the younger group for reasons of poor health, and two birds which had not yet recovered satisfactorily from surgery at the time of the first experiment were added to this group for the second experiment. Also, five of the younger birds which had been only partially hypophysectomized were studied in the second series of radioactivity counts. The older group was augmented by two hypophysectomized birds.

Most of the birds were killed either two months or five months after the radioactivity studies. The thyroids, adrenals, testes, and pituitary transplants were removed and weighed. The thyroids and pituitaries and the portions of the skulls including the sellae turcicae were prepared for microscopic examination. Sections of thyroids and pituitaries were cut at 10 microns; and every tenth section was mounted and stained with hematoxylin and eosin. After the sella turcica regions had been kept in Perenyi's decalcifying solution for ten days, they were sectioned at 15 microns. Every tenth section was mounted and stained with hematoxylin and eosin.

RESULTS

Hypophysectomy was manifested in regression of both primary and secondary sex characteristics. The only visible sex characteristic that was not completely suppressed in the hypophysectomized birds was development of spurs. Birds hypophysectomized at the age of two months developed short spurs; birds hypophysectomized at three months developed longer spurs. Particularly dramatic changes occurred in the combs of the operated birds. Loss of color, shrinkage, and dryness became evident during the second or third post-operative week.

At about this same time a slow molt commenced. The new feathers that began to appear several weeks later were abnormally loose in texture, giving the birds a disheveled appearance. Nalbandov and Card (1943) have attributed this peculiar feather texture of hypophysectomized birds to suppression of barbule formation.

More subtle changes in the hypophysectomized birds appeared in their behavior. Even after full recovery from surgery, the hypophysectomized birds did not regain their erstwhile alertness and responsiveness. They did not try to escape handling and did not even make the sounds of alarm characteristic of normal birds. When hypophysectomized birds were liberated from their cages, they appeared dis-oriented and walked awkwardly.

Growth continued at an almost-normal rate after hypophysectomy, but the addition of fat was a disproportionately large component of this postoperative growth.

In the present experiments nine hypophysectomized birds had pituitary transplants in the anterior chambers of their eyes. These birds exhibited the outward signs of hypophysectomy described above.

The radioactivity experiments revealed a considerable difference between hypophysectomized and control birds of the younger group in their ability to accumulate I^{131} in their thyroid glands (Figs. 1 and 3). The thyroids of the controls were more radioactive than the thyroids of the hypophysectomized birds at all the counting times in both experiments. Also, the steep rise in radioactivity of the control thyroids beyond the fifth hour after the birds had been injected with I^{131} was not paralleled by an increase in the radioactivity of the thyroids of the hypophsectomized birds.

At each counting time, the range in the counts for the partially hypophysectomized birds far exceeded the range in the counts for the other two groups (Fig. 3), extending from a level above the highest of the control counts to the

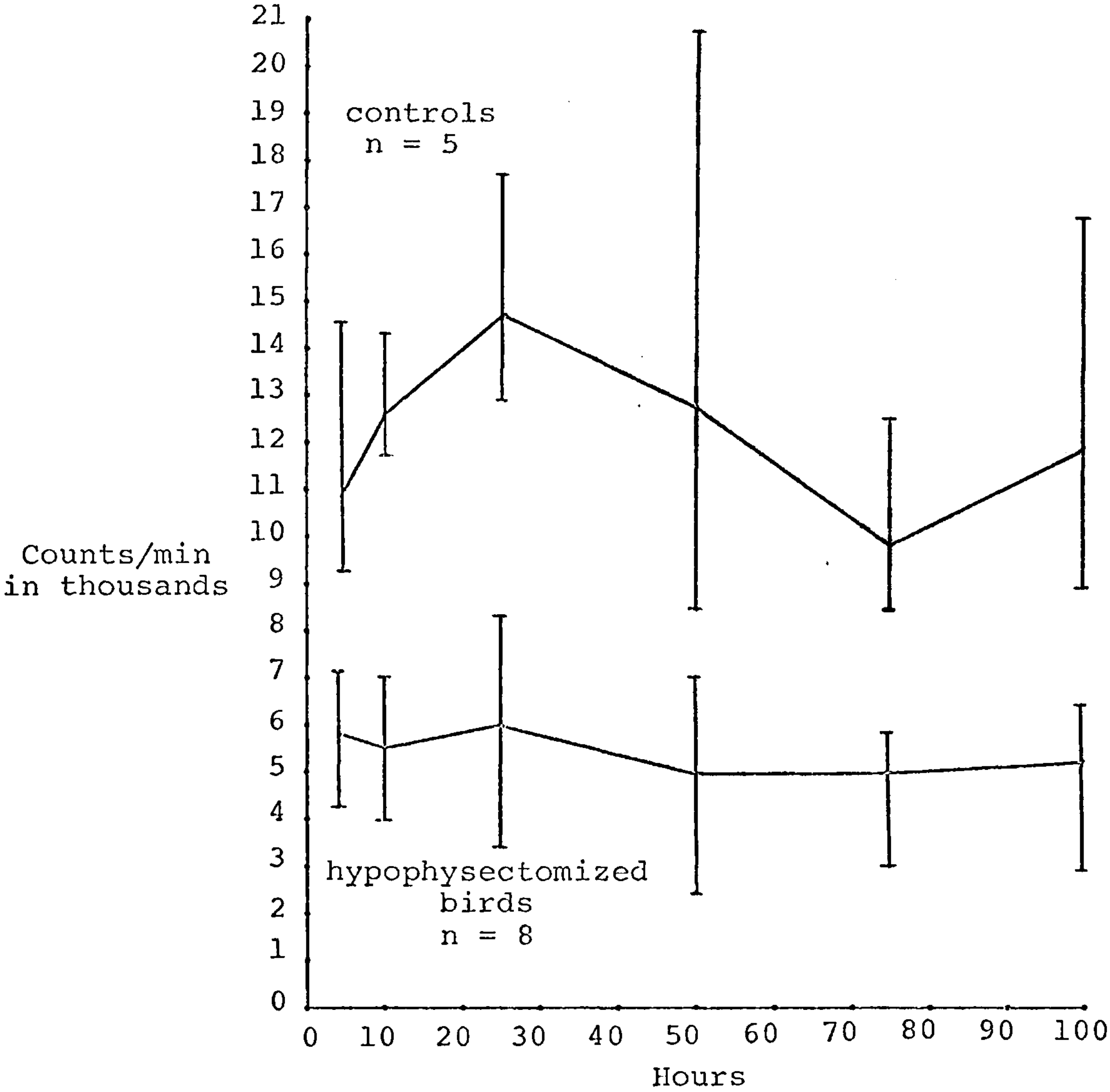

Figure 1. Average thyroid radioactivity counts I, first experiment.

Age of birds = 3 months; time since hypophysectomy = 1 month; vertical bars represent range.

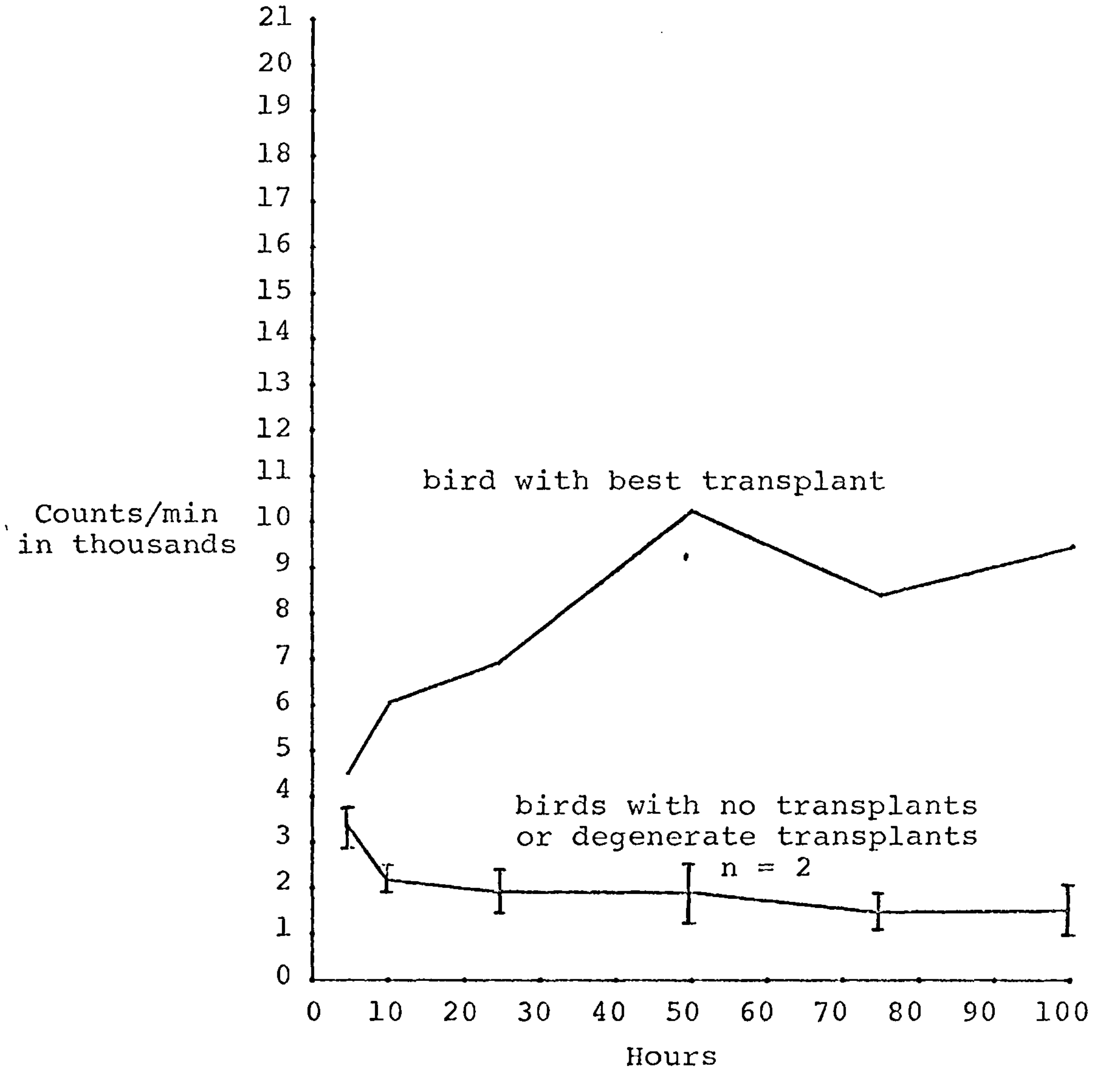

Figure 2. Average thyroid radioactivity counts II, first experiment.

Age of birds = 6 months; time since hypophysectomy = 3 months; vertical bars represent range.

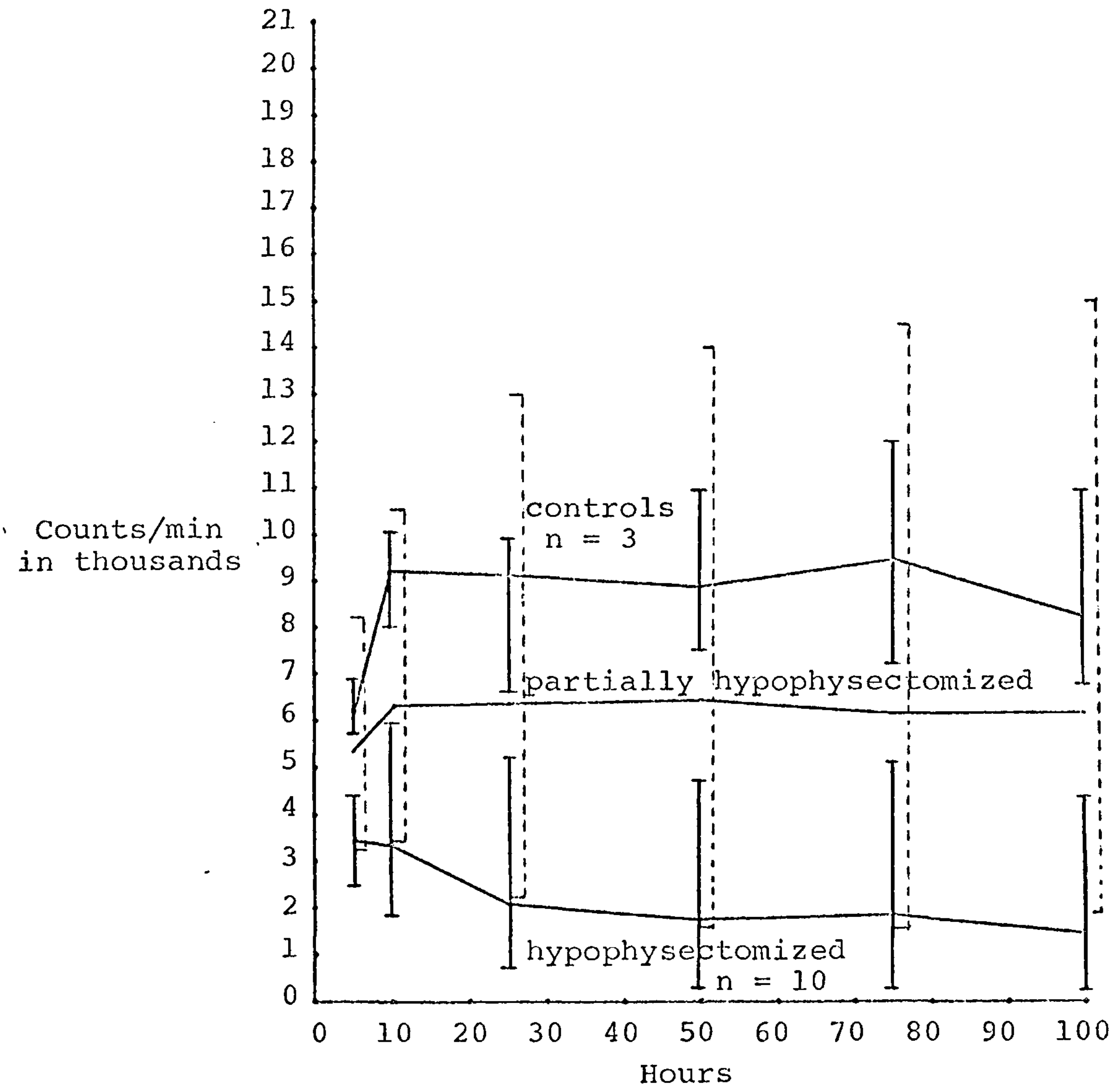

Figure 3. Average thyroid radioactivity counts I, second experiment.

Age of birds = 5 months; time since hypophysectomy = 3 months; vertical bars represent range.

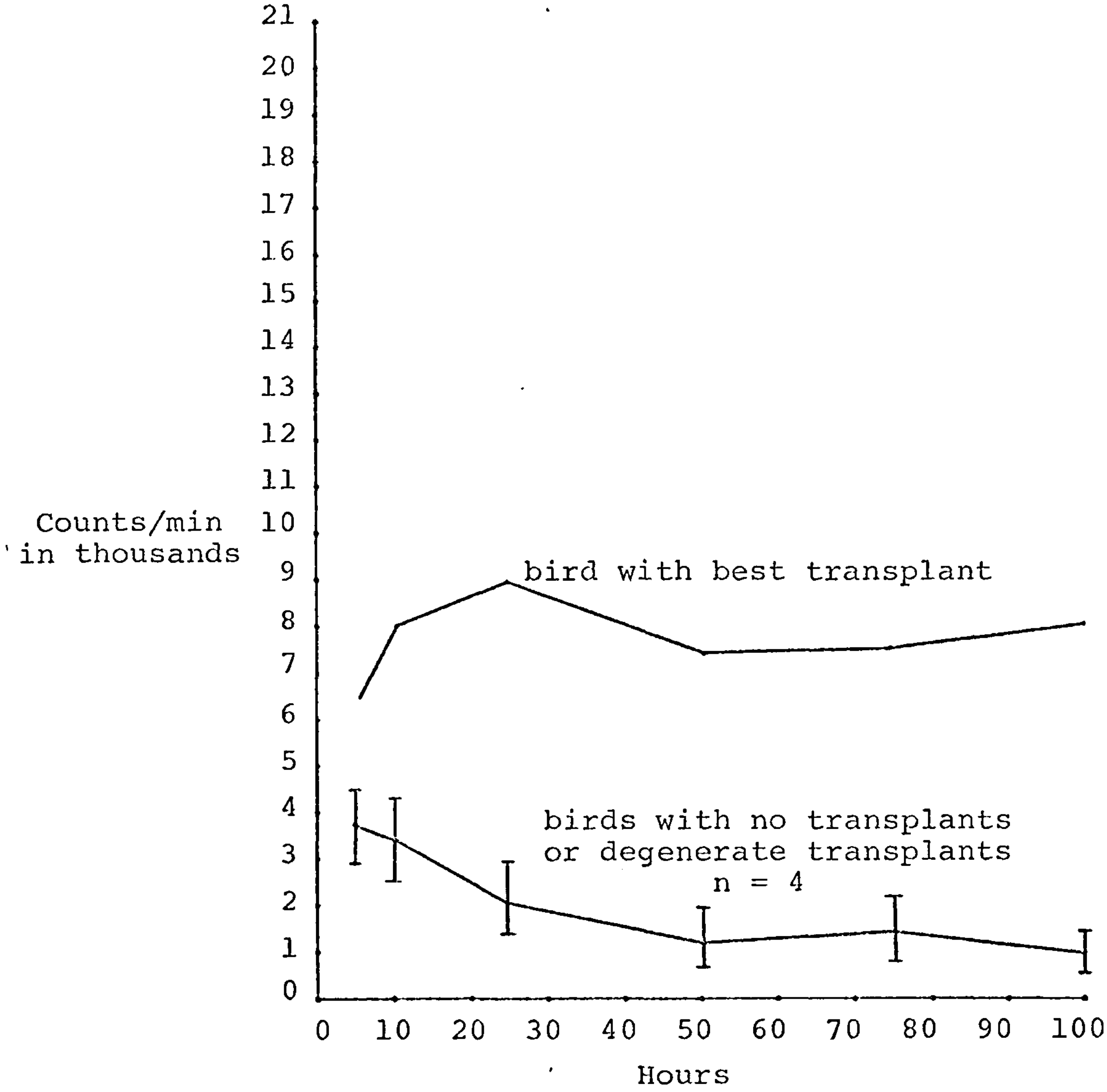

Figure 4. Average thyroid radioactivity counts II, second experiment.

Age of birds = 8 months; time since hypophysectomy = 5 months; vertical bars represent range.

level of the average of the counts for the hypophysectomized birds.

Although the older birds (represented in Figs. 2 and 4) exhibited generally lower thyroid radioactivity than the younger group in both experiments, the patterns of change in thyroid radioactivity were similar in the two groups. One of the older hypophysectomized birds proved to be an exception. This bird differed widely from all the other hypophysectomized birds of both groups and corresponded closely to the controls of the younger group in both level of thyroid radioactivity and in the shape of the curve showing change in radioactivity with time (Figs. 2 and 4). Interestingly, the pituitary transplant which this bird carried had been maintained in a condition far superior to that of the transplants in the other birds.

Most of the transplanted pituitaries shrank to small fractions of their original sizes, and most of them also degenerated to some extent internally. Only one of the transplants maintained an apparently normal internal condition and at the same time escaped a drastic reduction in size. This transplant is the exception mentioned in the preceding paragraph. The bird carrying this transplant exhibited all the general visible signs of hypophysectomy, but thyroid radioactivity in this bird far surpassed that in all the other hypophysectomized birds at all counts, and the initial increase in thyroid radioactivity persisted longer in this bird than in any of the other hypophysectomized birds. In its thyroid-radioactivity characteristics, this bird was similar to the unoperated birds. With exception of this one case, no consistent differences in thyroid radioactivity appeared between hypophysectomized birds with pituitary transplants and those without transplants, and no correlation was discerned between size of pituitary transplant and level of thyroid radioactivity among the birds with transplants.

Examination of the thyroids at autopsy was bedeviled by the same difficulty that Nalbandov and Card (1943) bemoaned: "[The thyroids] become flat bandlike structures [by 10 to 20 days after hypophysectomy] and are so small that they can be found only with great difficulty. After more prolonged apituitarism when adiposity of the neck region becomes pronounced, it becomes sometimes impossible to locate these glands in the surrounding tissue." Accordingly, the attempt to determine the weights of the thyroids of the hypophysectomized birds failed. Thyroids were found in most of the birds, but the attempt to extricate them from the surrounding tissue resulted in most instances in removal of only parts of the thyroids or in removal of extraneous tissue with the thyroids or in both of these bungles. The tissue samples that were saved indicated a concomitance of reduced follicle size with the depression of activity revealed by the radioactivity counts.

There was a great gap between the hypophysectomized birds and the controls in the weight of the testes per unit total body weight. The ratio of this measure for the hypophysectomized birds to that for the controls approximated the 1:50 ratio that Rosenberg, Dimick, and La Roche (1963) reported. The testes of the partially hypophysectomized birds weighed only slightly less in relation to the entire body than did the testes of the controls.

Though the thyroids and testes of the hypophysectomized birds were very mucy smaller than those of the controls, the adrenal glands of the hypophysectomized birds weighed on the average more than half as much as the adrenal glands of the controls. This observation also concurs with the report of Rosenberg et al. (1963). Two of the five partially hypophysectomized birds ranked with or very near the controls in adrenal weight per unit total body weight, and the other three ranked at or below the median for the hypophysectomized birds. The pattern of variation here was similar to that found for the variation in thyroid radioactivity of the partially hypophysectomized birds. However, the corresponding ranks were not occupied by the same individuals. The bird with the highest thyroid radioactivity ranked lowest among the five in adrenal weight per unit total body weight, and the bird with the lowest thyroid radioactivity ranked highest in adrenal weight per unit total body weight. The other three birds did not complete an inverse correlation pattern.

DISCUSSION

Complete hypophysectomy of the chicken is difficult to perform. Removal by suction is very likely to fragment the gland, and since the stalk and the capsule of the gland are left in place, fragments of the pituitary adhering to these structures escape removal. Most of the operations performed in this study left pituitary fragments in situ. However, in most instances, these fragments were nearly microscopic even five months or longer after hypophysectomy. During this long interval between hypophysectomy and autopsy, the pituitary fragments might well have grown from a much smaller size. It seems likely in view of what has been postulated about feedback control of pituitary activity that after hypophysectomy, deficiency of pituitary hormones and hormones of pituitary targets would have favored hypertrophy of any fragments that remained in the sella turcica. Where the fragments were very minute and where these minute fragments gave no signs of competency, the birds harboring them were labeled "hypophysectomized." Admittedly, this policy seems to rest in part on argumentum in circulo, the fact of functional hypophysectomy being judged by a few physiological signs and the signs having been established by association with the hypophysectomy. But really, the establishment of the symptoms of hypophysectomy was not done in the present study. Though this study reexamined some of these signs, it did not venture to postulate any new ones or to dispute any that had been set forth in earlier studies. The categorization of birds with very small pituitary fragments as functionally hypophysectomized was based on symptoms which Mitchell (1929); Nalbandov and Card (1943); and Rosenberg, Dimick, and La Roche (1963) had described for completely hypophysectomized birds.

Where pituitary fragments left in the sella turcica at hypophysectomy were macroscopically noticeable and where any evidence attributable to function of these fragments appeared, the birds carrying them were labeled "partially hypophysectomized."

The effects of autografting pituitaries into eyes were beclouded by the severe reductions in normal tissue which the transplants suffered. With the one exception already noted, none of the transplanted pituitaries gave any evidence of being functional. This lack of evidence might suggest that the eye is an unfavorable site for prompt revascularization of an extirpated pituitary or that the fragile pituitaries were traumatized beyond recovery in the transplantation. The latter conjecture seems reasonable in view of the difficulty encountered in the transplantation. The transplanted pituitary had a distressing propensity to float out through the incision in the cornea as the forceps with which it had been inserted was withdrawn. Usually, the pituitary had to be replaced into the eye several times before it finally stayed behind when the forceps was withdrawn. This procedure inevitably did violence to the gland.

Disconnection of the pituitary from the hypothalamo-hypophyseal blood vessels or even removal from the sella turcica does not by itself abolish all functions of the pituitary. Shirley and Nalbandov (1956) transected pituitary stalks with small plastic plates and left these in place to keep the pituitaries isolated from the hypothalami. The sex characteristics of the birds thus treated were disrupted, but the thyroids and adrenals continued their normal functions. Pituitaries transplanted to kidneys by Ma (1963) continued to produce TSH and ACTH normally but ceased producing gonadotropins. Rosenberg, Dimick, and La Roche (1963) observed the same results of transplantation to kidneys. The bird which in the present study maintained the pituitary transplant in its eye in the best condition likewise appeared normal in its thyroid and adrenal maintenance though its sex characteristics regressed in the manner typical of apituitarism. Thyroid and adrenal stimulation by the pituitary thus seem to be less dependent upon hypothalamic influence than gonadal stimulation appears to be.

The evidence that this study adduces for the suggestion that a pituitary transplanted into the eye continues to influence the targets of its trophic hormones is very tenuous at best, coming as it does from a single bird. And it would be a herculean undertaking to obtain an adequate number of birds like this one unless the rate of survival of pituitary tissue transplanted into the eye could be vastly improved over that which prevailed in this endeavor. However, if a pituitary autografted into the eye cannot be preserved from severe atrophy, perhaps the test of efficacy of pituitary transplanted to the eye could be done with homografts of an excess of tissue, which perhaps would not shrink to a size smaller than that of a normal pituitary.

Gross inequality in size between the ocular transplants and the pituitaries left intact interferes with assessment of the effects of transplantation, to be sure. But perhaps comparison of the transplants with the pituitary fragments left in place in the partially hypophysectomized birds would be germane to the problem. Some of the transplants comprised more normal pituitary tissue than the fragments left _in situ_ in the partially hypophysectomized birds; yet the indices of pituitary function used in this study revealed at least some pituitary effectiveness in all of the partially hypophysectomized birds but none in the birds with transplanted pituitaries only (with the same exception cited in the preceding paragraphs). It might therefore be suggested that pituitary tissue in the anterior chamber of the eye does not function in the same measure as an equal amount of pituitary left in the sella turcica in connection with the hypothalamo-hypophyseal vessels.

In partially hypophysectomized birds, pituitary fragments less than a quarter of the entire gland maintained normal thyroid structure and I^{131} concentration, normal adrenal weight, and normal sex characteristics. However, no individual among the five partially hypophysectomized birds maintained more than two of these three normal features. Thus it appears that fragments from different locations in the pituitary produce different pituitary hormones or produce the pituitary hormones in different amounts. For TSH production such localization has been definitely postulated by Mikami (1958). TSH, according to Mikami, is produced only in the cephalic lobe of the anterior pituitary. The present study suggests that production of ACTH might also be localized in the pituitary, but since all five of the partially hypophysectomized birds developed the normal sex characteristics, there is no evidence here for localization of gonadotropin production.

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