THE ROLE OF THE LIVER AND THE ADRENAL IN PRODUCING ELEVATED PLASMA 17-HYDROXYCORTICOSTEROID LEVELS IN SURGERY[1]

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(Submitted for publication April 7, 1954; accepted July 15, 1954)

In another paper of this series (1) it was demonstrated that most patients undergoing surgery developed increased plasma levels of 17-hydroxycorticosteroids. Evidence was presented that these rises are mediated in part by extra-adrenal factors. The concept that extra-adrenal factors may affect adrenal steroid levels is not a new one but little evidence to support such a concept has been presented.

The liver is known to contain enzyme systems capable of modifying these compounds *in vitro* (2). The reduced effect of the diseased liver in estrogen metabolism has been advanced by many authors as the explanation of the clinical evidence suggestive of estrogenic overactivity in patients with cirrhosis of the liver (3). That liver disease results in profound alteration of adrenocortical steroid metabolism also, has been demonstrated in current studies by Brown, Willardson, Samuels, and Tyler (4).

Thus it seemed logical to study the relation of hepatic function to the magnitude of the rise in 17-hydroxycorticosteroids after surgery. In addition, it was hoped that by the determination of free and conjugated steroids of this type in the urine an estimate of the total adrenal output of these substances would be obtained. By the correlation of the data it was expected that the influence of increased adrenal secretion and of impaired steriod disappearance resulting from hepatic dysfunction could be evaluated at least on a qualitative basis. In this report data dealing with these aspects of the effect of surgery on adrenal steroid metabolism will be presented and an attempt made to relate this information to the overall picture of the regulation of adrenal cortical secretion, insofar as this is possible on the basis of the evidence now available.

MATERIALS AND METHODS

Twenty relatively healthy patients who were to undergo elective surgery were selected so that the series would include those having major and minor procedures, both sexes and various ages. During the preoperative period a sulfobromphthalein (BSP) excretion curve was determined on each patient. Five mg. per kilo of BSP dye was injected intravenously within one minute. Blood was drawn before the dye injection and at 10- to 12-minute intervals afterward until four additional samples had been collected. The time of each sample was carefully noted. The serum BSP concentration was determined as mg. per 100 ml. of serum by a modification of the Rosenthal method (5). (The usual clinical expression of dye concentration is in terms of per cent retention, which assumes that an injection of 5 mg. per kilo will produce a serum level of 10 mg. per 100 ml. arbitrarily taken as 100 per cent.) Analysis of these data was made by plotting the dye concentration as the ordinate on a logarithmic scale against time on the abscissa. In normal individuals straight lines were obtained by this technique and the rate of clearance could be calculated easily. From the graphs accurate estimation of the 45-minute retention could also be made. Between the first and second hour after surgery a second BSP excretion study was performed in the same fashion on each patient. Many of these curves differed from the control observations in that straight lines were not obtained when the data were plotted. The initial clearance rates were frequently more rapid than those found during the latter half of the 45-minute period. As a result, valid expression of the data as clearance rates was not possible. They are therefore recorded here in the usual clinical fashion as per cent dye retention at 45 minutes.

Heparinized plasma for 17-hydroxycorticosteroid determination was obtained from each patient at 8 A.M. on one or more days pre-operatively. Similar samples were drawn at two, four, six, and eight hours post-operatively on each patient. Plasma 17-hydroxycorticos-

[1] This investigation was supported in part by research grants from the National Institutes of Health, U. S. Public Health Service and from Armour and Co. and in part by a contract (DA-49-007MD-136) between the University of Utah and the Department of the Army.

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teroids were determined by a modification of the method of Eik-Nes, Nelson, and Samuels (6).

In eight representative patients of the group, 24-hour urine collections (7 A.M. to 7 A.M.) were made on a control day and on the day of the operation. The creatinine content of each sample was determined to indicate the completeness of the collection. In each sample the free and total 17-hydroxycorticosteroids were estimated by a technique previously described (4).

RESULTS

In Table I the patients have been listed in descending order of the highest steroid level observed post-operatively. The sex and age of each patient, the nature and duration of the operation, the anesthesia used, the post-operative condition and BSP retention pre- and post-operatively, are recorded as well as the steroid levels observed. The average control 17-hydroxycorticosteroid level of 11.7 μg per 100 ml. of plasma, was similar to that found in normal individuals (7). It will be noted that the increase in BSP retention post-operatively was higher in those patients who showed the higher steroid rises. This is illustrated graphically in Figure 1. Both values also show some correlation with the duration of the operation.

Patients Nos. 5 and 14 showed considerably more BSP retention in relation to the steroid rise observed than the remainder of the group. Patient No. 14 had failed to excrete BSP normally before operation; very probably the large post-operative increase is not as significant as an index of the degree of additional liver dysfunction as a similar increase in patients with initially normal liver function would be. It may be important that both of these patients had hypertensive heart disease and minimal congestive heart failure at the time of operation.

In six of the twenty patients in this study the plasma 17-hydroxycorticosteroids did not rise above 23 μg per 100 ml. of plasma, which represents the approximate upper limit of normal values at 8 A.M. However, because of the characteristic diurnal curve of these substances and the fact that the postoperative values extended into the afternoon, only the last two patients can be said to have failed to show a significant elevation of the steroid level. None of these six patients showed a significant increase in BSP retention post-operatively. In three additional patients (Nos. 9, 11, and 13) only a small increase in BSP retention was observed post-operatively and even these values were within the usually accepted normal range. All three showed moderate increases in 17-hydroxycorticosteroid levels. Patient No.

TABLE I

The effects of surgery on BSP retention and plasma levels of 17-hydroxycorticosteroids in twenty patients

Pt. No.	Sex and age	Operation	Duration (*hrs.*)	Anesthetic	Condition post-op.	% BSP retention at 45′ Pre-op.	% BSP retention at 45′ Post-op.	% BSP retention at 45′ Diff.	17-OH steroid plasma level Controls average	(γ/100 ml.) 2	4	6	8
1	F 60	Cholecystectomy	3	Ether	Shock	7	32	25	8	31	40	60	100
2	M 75	Herniorrhaphy	2	Spinal	Good	6	18	12	20	100	52	62	66
*3	M 61	Gastric resection	3½	Cyclo	Good	0	18	18	12	39	40	53	26
4	M 78	Perineal prostatectomy	1¼	Spinal	Good	0	29	29	13	31	45	52	52
*5	F 68	Bilat. vein ligation	1½	Spinal	Ht. failure	10	50	40	13	45	25	21	19
*6	M 69	Herniorrhaphy	1¾	Spinal	Good	10	20	10	10	30	44	40	40
*7	M 53	Gastric resection	3½	Cyclo	Good	3	21	18	11	34	39	44	30
8	M 47	Bilat. vein ligation	1½	Spinal	Good	10	23	13	12	37	32	14	17
9	M 25	Submucous resection	2	Local	Good	2	8	6	17	25	24	33	†
10	F 51	Submucous resection	1	Local	Good	16	26	10	9	9	31	12	10
11	M 49	Fusion of knee	1¾	Cyclo	Shock	0	5	5	12	25	28	27	31
12	M 77	Suprapubic prostatectomy	1½	Spinal	Good	2	16	14	9	30	26	26	19
*13	F 36	Tibial bone graft	2	Spinal	Good	3	9	6	14	22	12	27	19
*14	F 55	Pin removal	1½	Cyclo	Lryngl. sp. ht. failure	30	51	21	12	20	21	27	27
15	M 47	Sequestrectomy	1¼	Spinal	Good	1	1	0	9	12	20	20	23
16	M 28	Tendonotomy	1½	Pento	Good	5	8	3	18	7	10	20	20
17	M 40	Resection of toe	1	Local	Good	10	12	2	15	19	16	19	16
*18	F 20	Herniorrhaphy	1½	Spinal	Good	4	4	0	23	18	†	16	16
*19	M 49	Hemorrhoidectomy	½	Caudal	Good	2	4	2	9	3	6	8	7
20	M 14	Vasectomy	1	Local	Good	5	5	0	11	7	7	7	7

* Pre- and post-operative studies of urinary 17-hydroxycorticosteroids.
† Samples lost.

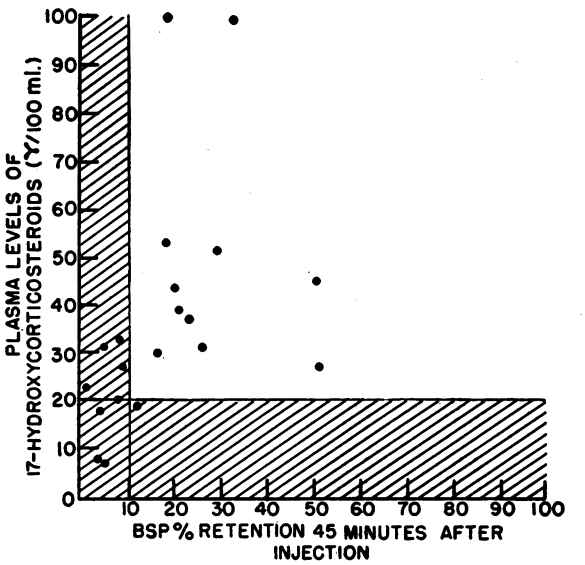

FIG. 1. RELATION OF POST-OPERATIVE INCREASE IN BSP RETENTION AT 45 MINUTES TO THE MAXIMAL PLASMA 17-HYDROXYCORTICOSTEROID LEVEL OBSERVED DURING THIS PERIOD

11 developed mild shock after the post-operative BSP determination had been completed and patient No. 9 was emotionally disturbed and excited to an unusual degree during the first few hours following his submucous resection.

The character of the data obtained is demonstrated more clearly in Figure 2. Here the findings in two representative patients (No. 3 and No. 19) are recorded in detail. They show the correlation of impaired BSP excretion and the magnitude of the post-operative 17-hydroxycorticosteroid rise.

The urinary excretion of free and conjugated 17-hydroxycorticosteroids by the eight patients in whom the observations were made is recorded in Table II. Again they are listed in the order of maximal plasma steroid level observed post-operatively. The control values fell within the range of values which have been observed in normal individuals (4). During the day of operation a rise in steroid excretion was observed in each patient except No. 19. He also failed to show a change in BSP excretion or a significant rise in plasma steroid levels post-operatively. While the others showed significant increases in urinary 17-hydroxycorticosteroid excretion, there was no obvious correlation between the magnitude of that rise and the degree of change in BSP clearance or plasma steroid level.

The excretion of free 17-hydroxycorticosteroids was only a small fraction of the total output. However, with the exception of patient No. 14, the relative increase in this fraction was larger than the increase in the total excretion of steroids. This would either indicate that the relative efficiency of conjugation was reduced or that renal clearances of the free and conjugated compounds are dissimilar.

DISCUSSION

We have presented evidence that the magnitude of the rise in plasma 17-hydroxycorticosteroids following surgery shows some correlation with the increase in BSP retention occasioned by this trauma and with the duration and severity of the surgical procedure. Although the total, free, and conjugated 17-hydroxycorticosteroids in the urine showed an increase in all but one of the eight patients in whom they were determined, their

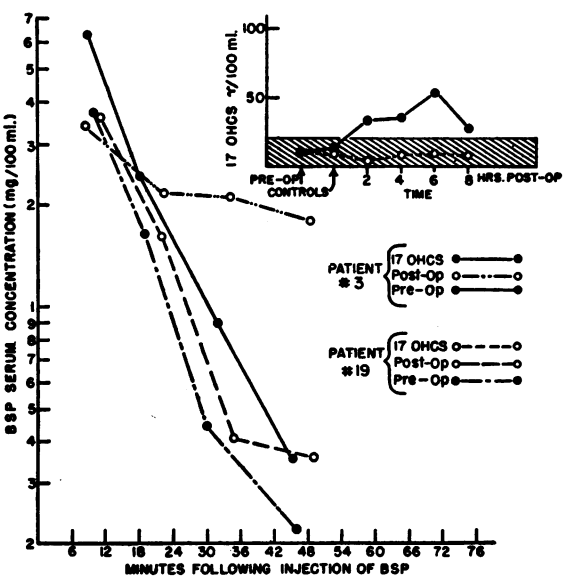

FIG. 2. DETAILED BSP AND 17-HYDROXYCORTICOSTEROID (17-OHCS) CURVES OBSERVED IN TWO REPRESENTATIVE PATIENTS

Note that no elevation of steroid level occurred post-operatively in patient No. 19 and that he excreted BSP essentially as well post-operatively as pre-operatively. In contrast, patient No. 3 had a marked rise in 17-OHCS levels and showed impairment of BSP excretion post-operatively.

TABLE II

Urinary 17-hydroxycorticosteroids in eight patients undergoing surgery

Patient No.	Collection	17-Hydroxycorticosteroids			Maximal plasma level 17-hydroxycorticosteroids
		Free mg./24 hr.	Conj. mg./24 hr.	Total mg./24 hr.	γ/100 ml.
3	Pre-op.	.15	2.1	2.25	13
	*Post-op.	.99	8.0	8.99	53
5	Pre-op.	.17	4.5	4.67	13
	*Post-op.	.52	5.6	6.12	45
6	Pre-op.	.18	2.4	2.58	11
	*Post-op.	.71	8.9	9.61	44
7	Pre-op.	.15	2.0	2.15	12
	Post-op.	1.5	6.4	7.9	39
13	Pre-op.	.16	4.9	5.06	17
	Post-op.	.87	10.8	11.67	27
14	Pre-op.	.40	5.6	6.0	12
	Post-op.	.43	9.1	9.53	27
18	Pre-op.	.11	4.4	4.51	23
	Post-op.	.43	8.0	8.43	18
19	Pre-op.	.25	5.6	5.85	10
	Post-op.	.36	4.8	5.16	8

* Collection incomplete.

magnitudes seemed to have no uniform relation to the other factors.

Certain limitations in the interpretation of our observations should be emphasized. First, the relation of the change in BSP excretion to the rise in plasma levels of adrenocortical steroids offers only presumptive evidence that functional impairment of the liver plays a role in the observed steroid changes. Second, the BSP clearance measures two factors: the rate of hepatic blood flow and the ability of the liver cells to excrete the dye. There is some evidence to suggest that the excretory function of the liver is a biphasic phenomenon in which the dye is first concentrated, possibly by the Kuppfer cells, and then excreted in the bile by an additional mechanism (8). The biphasic character of the post-operative excretion patterns which we have observed would seem to confirm this hypothesis. In addition, impaired ability of the liver to excrete BSP, even though that process involves multiple mechanisms, probably involves different systems than those concerned in the removal of corticosteroids from the circulation. Nevertheless, some evidence of a relation between these mechanisms in the usual clinical varieties of liver disease has been demonstrated by Brown, Willardson, Samuels, and Tyler (4). It is possible that there is a similar relationship in the more acute hepatic impairment which was studied here.

It may be added that a number of previous observers have pointed out that a decrease in liver function ocurs during and following anesthesia (9) and/or surgical procedures (10–12). They have shown that the severity of the trauma is related to the degree of liver impairment. This tends to confirm the significance of the changes observed in our own relatively small series.

A third limitation is concerned with the time relationships involved. For technical reasons plasma 17-hydroxycorticosteroids were determined at intervals over an eight-hour period while BSP clearance was studied during only a 45-minute interval in that period. Thus if, as seems probable, very rapid changes are occurring in hepatic function the BSP value obtained may not represent the average situation during the eight-hour period. Another discrepancy in time is the use of 24-hour urine collections, which was dictated not only by clinical convenience but also because of the delay in the excretion of steroids and the

marked diurnal variation in their rate of excretion under basal conditions (13).

In spite of these limitations the correlations seem evident. Because of these limitations, however, more significance must be given in the interpretation of the data to the qualitative character of the changes than to their actual magnitude.

Thorn, Jenkins, Laidlaw, Goetz, and Reddy (14) have reported that the total urinary adrenal corticosteroids are increased after major operative procedures but they did not examine the free and conjugated fractions in their studies. The magnitudes of the changes which they observed are similar to those reported here. They also observed that minor operative procedures and relatively mild environmental "stresses" did not produce an increase in the urinary excretion of total 17-hydroxycorticosteroids. Their observations, as well as those presented here, would seem to confirm our previous conclusion (1) that increased adrenal secretion contributes to the elevation of the plasma steroid level during and following major surgical procedures.

A review of the factors which are now known, or are thought, to be concerned in the metabolism of adrenal steroids may help in understanding the significance of the observations presented here and should point out the areas in which problems remain.

As is diagrammatically represented in Figure 3, a number of factors are concerned in the regulation of circulating adrenal cortical steroid levels. On the positive side the secretion of the adrenal cortex is apparently of primary importance. As we have demonstrated (15), the peripheral blood level depends, at least in part, on the rate at which ACTH reaches the gland. There is, however, a maximal response which cannot be exceeded by increasing the concentration of ACTH. This level probably is determined by the maximum rate of sustained secretion by the adrenal gland.

As we go clockwise about the diagram the next factor is related to the tissues other than the liver and kidney. At present it is impossible to measure the amounts of adrenal steroids in these tissues, their rate of exchange or the speed of their destruction. Previous attempts to demonstrate tissue removal of 17-hydroxycorticosteroids by studies of arteriovenous differences across the femoral

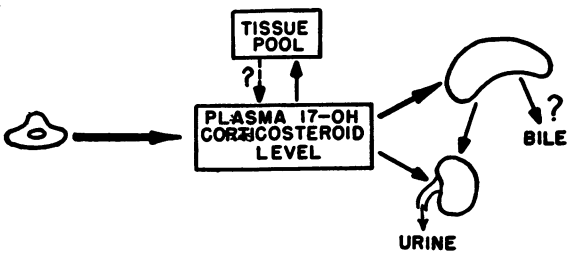

FIG. 3. DIAGRAMMATIC REPRESENTATION OF THE FACTORS AFFECTING PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS

circulation both in normal and in inflamed extremities have been unsuccessful (16). The rate of removal and of alteration of the steroids in the tissues must, therefore, presumably be less than the limits of measurement available for such a study at present.

On the other hand, current and previous studies in our laboratory have demonstrated a significant decrease in plasma levels across the hepatic circulation (17). Thus it would appear that the liver is the main site of removal of the 17-hydroxycorticosteroids which are measured by the procedure used in these studies. Renal excretion of steroids in the free form is extremely small except when the plasma levels are greatly elevated. Moderate amounts appear in conjugated form after oral or parenteral administration. This fraction never exceeds approximately one-third of the administered steroid and is usually very much less than this (13). These conjugates presumably are formed in the liver and are not measured by our plasma method.

These considerations indicate that the main factor concerned in the regulation of plasma levels, other than adrenal secretion, is the liver. The correlations which are presented in this report are consistent with the hypothesis that hepatic removal of steroids from the circulation is an important factor in determining the peripheral plasma level. As discussed above, the techniques which we have used are not adequate to distinguish between changes in blood flow and functional damage to the liver cells but the persistent impairment of BSP excretion for a period of days post-operatively, which has been shown by others, suggests that the latter occurs. Evidence of impaired liver blood flow has also been presented and it seems

probable that both factors are involved during the immediate post-operative period which we have studied. It seems to us that the effect of the liver must be considered whenever conclusions are drawn concerning adrenal secretion either from data based on metabolic changes or from steroid levels.

Persistent adrenal secretion at a greater-than-normal rate presumably resulting from endogenous ACTH, at a time when the steroid level is already elevated would seem to argue against the hypothesis presented by Sayers and Sayers (18) that the steroid level regulates the output of ACTH. The observed diurnal variation in normal men and the rather wide variability of normal levels at any given time also have been interpreted as evidence against a homeostatic mechanism (7). However, the consistency of the average value in normals, in patients with liver disease where impaired removal has been demonstrated (4), and in a wide variety of chronic illnesses (19) would seem to indicate that there is a homeostatic mechanism which responds less rapidly than that controlling certain other substances, such as the blood sugar level, but none-the-less functions quite effectively over longer intervals. In addition, there must be a mechanism which calls forth increased secretion acutely in the presence of severe trauma such as a major surgical procedure.

SUMMARY

1. After surgical operations the magnitude of the plasma 17-hydroxycorticosteroid levels attained shows a correlation with impaired BSP excretion and the length and severity of the operative procedure.

2. Conjugated urinary 17-hydroxycorticosteroids increased after all except one brief, minor procedure but showed no correlation with the plasma levels attained.

3. The factors which affect the plasma levels are discussed.

4. The hypothesis is proposed that the increased plasma levels of 17-hydroxycorticosteroid after surgery are the result of both increased adrenal secretion of these steroids and impaired hepatic removal.

ACKNOWLEDGMENTS

We are grateful to Dr. E. E. Hays of Armour and Company for generous supplies of ACTH, and to Smith, Kline and French for part of the BSP used. We wish to extend special thanks to the members of the Departments of Surgery and Anesthesia for their wholehearted cooperation in making this study possible.

REFERENCES

1. Sandberg, A. A., Eik-Nes, K., Samuels, L. T., and Tyler, F. H., The effects of surgery on the blood levels and metabolism of 17-hydroxycorticosteroids in man. J. Clin. Invest., 1954, 33, 1509.
2. Samuels, L. T., The Metabolism of the Steroid Hormones in Chemistry of Fats and Lipids, London, Pergamon Press, 1954, vol. 3 In press.
3. Lloyd, C. W., and Williams, R. H., Endocrine changes associated with Laennec's cirrhosis of the liver. Am. J. Med., 1948, 4, 315.
4. Brown, H., Willardson, D. G., Samuels, L. T., and Tyler, F. H., 17-Hydroxycorticosteroid metabolism in liver disease. J. Clin. Invest., 1954, 33, 1524.
5. Gaebler, O. H., Determination of bromsulphalein in normal, turbid, hemolyzed or icteric serums. Am. J. Clin. Path., 1945, 15, 452.
6. Eik-Nes, K., Nelson, D. H., and Samuels, L. T., Determination of 17,21-hydroxycorticosteroids in plasma. J. Clin. Endocrinol. & Metab., 1953, 13, 1280.
7. Bliss, E. L., Sandberg, A. A., Nelson, D. H., and Eik-Nes, K., The normal levels of 17-hydroxycorticosteroids in the peripheral blood of man. J. Clin. Invest., 1953, 32, 818.
8. Mendeloff, A. I., Some mechanisms involved in testing hepatic excretory function. Bull. New England M. Center, 1949, 11, 163.
9. Shackman, R., Graber, I. G., and Melrose, D. G., Liver blood flow and general anesthesia. Clin. Sc., 1953, 12, 307.
10. Tagnon H. J. Robbins, G. F., and Nichols, M. P., The effect of surgical operations on the bromsulfalein-retention test. New England J. Med., 1948, 238, 556.
11. Habif, D. V., Papper, E. M., Fitzpatrick, H. F., Lowrence, P., Smythe, C. McC., and Bradley, S. E., The renal and hepatic blood flow, glomerular filtration rate, and urinary output of electrolytes during cyclopropane, ether, and thiopental anesthesia, operation, and the immediate post-operative period. Surgery, 1951, 30, 241.
12. Schmidt, C. R., Unruh, R. T., and Chesky, V. E., Clinical studies of liver function. I. The effect ot anesthesia and certain surgical procedures. Am. J. Surg., 1942, 57, 43.

13. Sandberg, A. A., Nelson, D. H., Glenn, E. M., Tyler, F. H., and Samuels, L. T., 17-Hydroxycorticosteroids and 17-ketosteroids in urine of human subjects: Clinical application of a method employing β-glucuronidase hydrolysis. J. Clin. Endocrinol. & Metab., 1953, **13**, 1445.
14. Thorn, G. W., Jenkins, D., Laidlaw, J. C., Goetz, F. C., and Reddy, W., Response of the adrenal cortex to stress in man. Trans. A. Am. Physicians, 1953, **66**, 48.
15. Eik-Nes, K., Sandberg, A. A., Nelson, D. H., Tyler, F. H., and Samuels, L. T., Changes in plasma levels of 17-hydroxycorticosteroids during the intravenous administration of ACTH. I. A test of adrenocortical capacity in the human. J. Clin. Invest., 1954, **33**, 1502.
16. Nelson, D. H., Samuels, L. T., Willardson, D. G., and Tyler, F. H., The levels of 17-hydroxycorticosteroids in peripheral blood of human subjects. J. Clin. Endocrinol., 1951, **11**, 1021.
17. Nelson, D. H., and Harding, B., Effect of liver on blood levels of 17-hydroxycorticosteroids in the dog. Federation Proc., 1952, **11**, 379.
18. Sayers, G., and Sayers, M. A., Regulation of pituitary adrenocorticotrophic activity during the response of the rat to acute stress. Endocrinology, 1947, **40**, 265.
19. Perkoff, G. T., Sandberg, A. A., Nelson, D. H., and Tyler, F. H., Clinical usefulness of determination of circulating 17-hydroxycorticosteroid levels. Arch. Int. Med., 1954, **93**, 1.