The Effect of Hypophysectomy and Growth Hormone Replacement on Albumin Synthesis and Catabolism in the Rat

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Albumin synthesis and catabolism were respectively measured by McFarlane's (1963) sodium [^{14}C]carbonate method and I-labelled albumin in hypophysectomized rats, both untreated and treated with growth hormone. Hypophysectomy resulted in a decrease in both albumin synthesis and catabolic rates. These changes as shown by pair-feeding experiments could not be ascribed to decreased food intake alone. Growth hormone was shown to partially restore both albumin synthesis and catabolic rates. It is proposed that growth hormone stimulates albumin synthesis and that its effect on albumin catabolism is secondary to changes in the mass of the intravascular albumin pool.

Hypoalbuminaemia and a decreased intravascular albumin mass are well recognized consequences of hypophysectomy (Enerbäck, Lundin & Mellgren, 1959; Levin & Leatham, 1942; Li & Reinhardt, 1947). The nature of the underlying changes in synthesis or catabolism have not been fully elucidated. Ulrich, Tarver & Li (1954) showed that 'synthesis-catabolism' was decreased in rats after hypophysectomy, and Jeejeebhoy, Boucher & Hartog (1965) found rates of 'synthesis plus transfer' and catabolism to be decreased in hypopituitary humans.

Studies of the effect of growth hormone on albumin metabolism have given variable results. Ulrich *et al.* (1954) believe growth hormone stimulates albumin synthesis, whereas Jeejeebhoy *et al.* (1965) and Gabuzda, Jick & Chalmers (1963) believe growth hormone slows albumin catabolism. The discrepancies in these studies are due in part to the use of isotopic techniques that do not entirely fulfil the requirements for valid measurement under conditions of metabolic change such as exist after hypophysectomy and hormonal treatment. It is now possible to measure albumin synthesis directly by the sodium [^{14}C]carbonate method (McFarlane, 1963; Reeve, Pearson & Martz, 1963) and to measure catabolism independently of the synthesis rate by means of I-labelled albumin (Matthews, 1961), both forms of measurement being valid in the non-steady state. By using the [^{14}C]carbonate method Sellers, Bonorris & Katz (1969) showed that growth hormone could stimulate albumin synthesis in the perfused rat liver, but Jeejeebhoy, Bruce-Robertson, Sadtke & Foley (1970) showed no effect on synthesis *in vivo* in normal

rats. In the hope of defining more clearly the changes occurring in albumin metabolism after hypophysectomy and after growth hormone treatment, the above techniques for measuring albumin synthesis and catabolism have been employed for the first time in a study on hypophysectomized rats and hypophysectomized rats receiving growth hormone-replacement therapy. Because the hypophysectomized rat shows a degree of anorexia, and the synthesis and catabolism of albumin are influenced by protein deprivation, the effect of sham operation and appropriate dietary restriction was assessed in a third group of rats by means of pair-feeding.

EXPERIMENTAL

Albumin catabolic rate studies. Albumin catabolic rate was measured in four groups of rats: (a) control group (16 rats); (b) hypophysectomized group (nine rats); (c) paired sham-operated group (five rats); (d) hypophysectomized group receiving growth hormone-replacement therapy (nine rats).

The 16 normal control rats were studied daily over a period of 8–10 days. In the three experimental groups of rats the tracer dose of albumin was injected and the pre-operative catabolic rate first established on a daily basis over a period of 3 or 4 days. These daily values were averaged to obtain a single average pre-operative value. Hypophysectomy or sham operation was then carried out and the fractional and absolute catabolic rate were determined daily for 9 days thereafter. The rats thus acted as their own controls both before the operation and throughout the 9-day study period, and it was also possible to check on the quality of the tracer by comparing the pre-operative catabolic rate with that of the normal control rats.

Albumin synthesis rate studies. The large amounts of plasma or blood required precluded serial synthesis rate determinations in the same rats as was possible when the catabolic rate was measured. Equal samples of blood or plasma from four rats were therefore pooled to provide each separate synthesis rate value.

Normal control values were obtained on nine such batches of rats (36 individual rats). In the three experimental groups synthesis rate was determined on days 2, 4, 6 and 9 post-operatively. On days 4 and 6 synthesis rate was measured in two batches of rats, but to permit statistical comparisons at the beginning and end of the study period, a total of six batches was studied on days 2 and 9.

Growth hormone injections (0.25 mg) were commenced 24 h before hypophysectomy and given daily until 24 h before the relevant synthesis rate measurement, or until 24 h before the completion of the catabolic rate study.

Animals. Male albino rats of the Wistar strain weighing 230–260 g were housed in single cages and fed on a standard rat-cube diet (Vereeniging Consolidated Mills Ltd., Cape Town, South Africa) containing 20% mixed protein.

Surgical procedures. (a) Hypophysectomy was performed under ether anaesthesia via the parapharyngeal route. The pituitary fossa was carefully checked at *post mortem* after the experiment for complete removal of the gland. (b) Sham operation was identical in all respects with the hypophysectomy, but the pituitary gland was left intact after drilling the base of the skull.

Materials. $^{131}\text{I}_2$ and $^{125}\text{I}_2$ were obtained from The Radiochemical Centre, Amersham, Bucks, U.K., as carrier-free preparations, free from reducing agent. Sodium [^{14}C]carbonate (specific radioactivity 20–40 mCi/mmol) was obtained from Phillips-Duphar, Petten, The Netherlands, as an aqueous solution. Bovine growth hormone (NIH-GH-B14) was supplied by the Endocrinology Study Section of the National Institutes of Health and had a mean potency of 1.04 U.S.P. units/mg and contained negligible amounts of prolactin, thyroid-stimulating, luteinizing and follicle-stimulating hormones.

Plasma albumin concentration. This was determined by the method of Fernandez, Sobel & Goldenberg (1966).

Cellulose acetate electrophoresis. This was carried out in 0.05 M-veronal buffer, pH 8.6.

Plasma urea concentration. This was determined on a Technicon Auto Analyser as described by Marsh, Fingerhut & Kirsch (1957).

Rat albumin. This was prepared by $(\text{NH}_4)_2\text{SO}_4$ precipitation (McFarlane, 1964).

Preparation of ^{125}I - or ^{131}I -labelled rat albumin. Iodination of rat albumin was performed by the iodine monochloride method (McFarlane, 1958). For catabolic rate studies ^{131}I was used. Iodination was followed by precipitation with $(\text{NH}_4)_2\text{SO}_4$ and dialysis against water (McFarlane, 1964), leaving more than 99% of the radioactivity bound to albumin. For synthesis rate measurements, albumin was labelled with either ^{125}I or ^{131}I . After iodination the sample was passed through a column of De-Acidite (Cl⁻ form) leaving 99% of the radioactivity bound to protein.

Plasma volume. This was determined by isotope dilution with ^{131}I - or ^{125}I -labelled rat albumin.

Rat growth hormone. This was measured by radioimmunoassay in a randomly selected group of animals for

9 days after hypophysectomy. Highly purified rat growth hormone (NIAMD-Rat GH-I-1, National Pituitary Agency) was iodinated by the method of Hunter & Greenwood (1962) and in addition served as reference standard. Monkey anti-(rat growth hormone) antibody was used in the assay, and separation of antibody-bound and free ^{131}I -labelled rat growth hormone complexes was achieved by chromatoelectrophoresis (Yalow & Berson, 1960).

Plasma corticosterone. Total 11-hydroxycorticoids in the plasma were measured by the method of Mattingly (1962). Since in the rat the corticosterone/cortisol ratio is approx. 7:1, total 11-hydroxycorticoids are hereafter for convenience referred to as plasma corticosterone.

Measurement of albumin catabolic rate. NaI (0.005%, w/v) was provided in the drinking water for 48 h before and for the duration of all catabolic rate studies. Urinary excretion of the label was measured indirectly by measuring the daily decrease in whole-body γ -radiation with a small whole-body counter. Plasma activity was assayed in a Packard Autogamma Spectrometer.

Approx. 20 μCi of ^{131}I -labelled rat albumin was administered intravenously into a tail vein under light ether anaesthesia. The appropriate radioactivity measurements were corrected for decay and dead time and the catabolic rate was calculated daily as described by Matthews (1961).

Measurement of albumin synthesis rate. The theoretical basis, rationale and assumptions of the sodium [^{14}C]carbonate method for the direct measurement of albumin synthesis have been presented elsewhere in detail (Koj & McFarlane, 1968; McFarlane, 1963; McFarlane, Irons, Koj & Regoez, 1965; Tavill, Craigie & Rosenoer, 1968). In the present study maximum specific radioactivity was derived and the fractional synthesis rate calculated as described by McFarlane *et al.* (1965), in which the fractional synthesis rate of urea was given by the slope of the semilogarithmic plot of the falling urea carbon specific radioactivity (c.p.m./mg of C) of endogenously produced [^{14}C]urea during the 6 h period of measurement and extrapolation of the graph back to zero time (t_0) gave the theoretical maximum specific radioactivity of urea C. The theoretical maximum specific radioactivity of albumin guanidine C (specific radioactivity at t_0) was derived by correcting the observed albumin guanidine specific radioactivity at the end of the 6 h period of measurement by a factor derived from the rate of disappearance from the intravascular compartment of an iodinated albumin tracer injected simultaneously with the sodium [^{14}C]carbonate.

Use of endogenous [^{14}C]urea results. The use of prelabelled urea for the definition of the plasma urea specific radioactivity curve is to be preferred over endogenously produced urea since continued incorporation of the ^{14}C into the latter results in an underestimate of the slope of the specific radioactivity curve (Jones, Craigie, Tavill, Simon & Rosenoer, 1968; Koj & McFarlane, 1968). The absence of facilities for the use of [^{13}C]urea and the particular experimental protocol chosen, necessitated the use of endogenous [^{14}C]urea data in the present study. The magnitude of the potential error associated with this approach has been assessed (Rothschild, Oratz, Mongelli & Schreiber, 1968; Jones *et al.* 1968) and does not appear to be significant.

Maximum albumin guanidine carbon specific radioactivity. Practical considerations governed the choice of the particular method described above for correcting for [^{14}C]albumin losses, as has been used by other workers (Kirsch, Frith, Black & Hoffenberg, 1968; Rothschild *et al.* 1968; Tavill *et al.* 1968). A potential source of error may be introduced since allowance is not made for the gradual arrival of [^{14}C]albumin molecules in the plasma (Koj & McFarlane, 1968).

Under light ether anaesthesia approx. 200 μCi of sodium [^{14}C]carbonate and 2 μCi of ^{125}I - or ^{131}I -labelled rat albumin were administered simultaneously by intravenous injection at the base of the tail. After 10 min the rats were bled from the tip of the tail for determination of the plasma volume, and at half-hourly intervals from 3 h to 6 h samples of blood were obtained for the determination of urea C specific radioactivity. At 6 h the rats were lightly anaesthetized with ether and bled by cannulating the aorta. Albumin guanidine carbon specific radioactivity and albumin and urea concentrations were determined on this final sample of plasma. The radioactivities of samples of plasma from the 10 min and final bleedings were counted to provide the correction factor for the albumin guanidine carbon specific radioactivity.

Once post-mortem examination had revealed the absence of pituitary-gland tissue the appropriate blood and plasma samples from four rats were pooled and processed as described by McFarlane (1963), McFarlane *et al.* (1965) and Tavill *et al.* (1968).

Urea carbon specific radioactivity. This was measured on deproteinized blood. Enzymically released CO_2 after treatment with urease was measured manometrically, condensed into phenylethylamine and assayed for radioactivity in a Beckman Liquid-Scintillation Counter.

Albumin guanidine carbon specific radioactivity. This was measured on albumin extracted from plasma with trichloroacetic acid and ethanol (Korner & Debro, 1956). The amino acid residue after acid hydrolysis was treated successively with arginase and urease to produce CO_2 , further processing being identical with that described above for urea carbon specific radioactivity measurement.

Statistical methods. The 'significance of the difference' between the various sets of experimental data was tested by means of the Mann-Whitney U test (Siegel, 1956). Wherever possible exact probabilities have been provided. Where sample sizes precluded this, an observed value for U ($U_{\text{obs.}}$) is presented together with a critical value of U at one of three significance levels. Where $U_{\text{obs.}}$ is less than or equal to critical U , the difference is significant at the particular significance level quoted and is written ($U_{\text{obs.}} < \text{critical } U$). Significance was assessed at the following levels: $P < 0.05$, probably significant; $P < 0.01$, significant; $P < 0.001$, highly significant.

RESULTS

Food consumption. The average quantities of food consumed daily by the normal control rats and by the three experimental groups of rats are presented in Fig. 1. At all times the daily intake of the three experimental groups of rats is well below the average normal intake. As the hypophysectomized rats (eating *ad libitum*) recovered from the

Fig. 1. Post-operative food consumption. The hatched area is the mean $\pm$ S.E.M. of the daily food consumption of 40 normal rats observed for a period of 2 weeks. —, Combined graph for hypophysectomized and sham-operated pair-fed rats; ----, graph for hypophysectomized rats treated with growth hormone. Values for untreated and treated hypophysectomized rats were derived from 21 animals observed daily for the 9 day study period. Experimental values are expressed as the means $\pm$ S.E.M.

effects of the operation, larger quantities of food were consumed. Bovine growth hormone appears to have no significant effect on the anorexia present after hypophysectomy.

Disappearance of pituitary hormones after hypophysectomy. Biochemical confirmation of hypophysectomy was obtained in eight rats by measuring plasma growth hormone and corticosterone concentrations on days 2, 4, 6 and 9 post-operatively. Neither hormone was detectable in the plasma 48 h after the operation.

Albumin metabolism. The results are presented in Figs. 2 and 3. Absolute rates were not expressed in terms of body weight, since correction for change in body weight alone does not take into account the marked differences between the body composition of starved, hypophysectomized and growth hormone-treated animals (Lee & Ayres, 1936; Levin, 1944) on which the plasma volume is to a considerable extent dependent (Jeejeebhoy *et al.* 1970). Since absolute rates can at least in part be accounted for by changes in plasma volume, greater emphasis has been placed on fractional rates which are not thus affected. Statistical evaluations were made as follows. Fractional and absolute synthesis rate and catabolic rate in the three experimental groups were compared (a) with normal values (on days 2 and 9 for synthesis rate and throughout the experimental period for catabolic rate) and (b) with each other (on days 2 and 9 only).

Fig. 2. Effect of (a) hypophysectomy, (b) sham operation and pair-feeding and (c) hypophysectomy plus growth hormone treatment on plasma albumin concentration and absolute synthesis and catabolic rates. The hatched areas represent the mean $\pm$ 2 s.d. of the values obtained from the normal control rats (for albumin concentration 60 rats, for catabolic rate 16 rats, for synthesis rate nine batches of four rats). The daily catabolic rate values for the three experimental groups of rats have been charted as the mean $\pm$ 1 s.d. The values presented for the albumin concentration are the serial values obtained in the catabolic rate studies, and are also charted as the mean $\pm$ 1 s.d. The values for the absolute catabolic rate and albumin concentration on days 4, 6 and 8 in the untreated hypophysectomized rats are not available, since it was thought that daily sampling might increase the post-hypophysectomy mortality rate. Later this precaution was found to be unnecessary, so that complete results are available for the growth hormone-treated rats.

Effect of hypophysectomy. (a) Albumin synthesis rate. Absolute and fractional synthesis rate fell sharply after hypophysectomy and 48 h post-operatively were both highly significantly less than normal (U 0<2 for both). The day 9 values displayed a further significant fall.

(b) Albumin catabolic rate. The absolute catabolic rate fell highly significantly on day 5 post-operatively (U 5<19). The fractional catabolic rate after a highly significant rise on day 2 (U 4<19) returned to normal values but continued to fall and became highly significantly less than normal on day 6 (U 10.5<19).

(c) Concentration of albumin. The plasma albumin concentration fell significantly below normal by day 7 (Fig. 2).

Effect of sham operation and pair-feeding. (a) Albumin synthesis rate. Absolute synthesis rate was at all times highly significantly less than normal values (U 0<2) but fractional rates were only probably significantly decreased (U 8.5<12) on day 2, whereas on day 9 there was no significant depression (U 16>12). The absolute synthesis rate

was significantly greater than that of the hypophysectomized group on days 2 and 9 (P 0.001 for both). The fractional synthesis rate on day 2 was only probably significantly greater (P 0.032), but on day 9 there was a highly significant difference (P 0.001).

(b) Albumin catabolic rate. For both absolute and fractional rates there was a highly significant rise on day 2 (U 0<5), after which there was a return to the normal range for the duration of the study period. Fractional catabolic rate was always significantly greater (day 2, U 3<5; day 9, U 2<5) and absolute catabolic rate highly significantly greater than that of the hypophysectomized group (U 0<1 on days 2 and 9).

(c) Concentration of albumin. The plasma albumin concentration remained within normal limits (Fig. 2).

Effect of hypophysectomy plus growth hormone treatment. (a) Albumin synthesis rate. The absolute synthesis rate was at all times highly significantly less than normal (U 0<2), the fractional rate significantly less on day 2 (U 3<7) but highly

Fig. 3. Effect of (a) hypophysectomy, (b) sham operation and pair feeding and (c) hypophysectomy plus growth hormone treatment on plasma albumin concentration and fractional synthesis and catabolic rates. The details for Fig. 2 apply here except that the only result affected by the restriction on sampling in the hypophysectomized rats is the albumin concentration. Abbreviation: IVP, intravascular pool.

significantly less on day 9 ($U 0 < 2$). Absolute and fractional synthesis rate of the growth hormone-treated rats were always significantly greater than that of untreated hypophysectomized rats (absolute synthesis rate on days 2 and 9, $P 0.002$; fractional synthesis rate on day 2 $P 0.008$, day 9 $P 0.004$). On day 2 the absolute synthesis rate ($P 0.090$) and fractional synthesis rate ($P 0.350$) were not significantly different from those of the sham-operated and pair-fed rats. On day 9 the absolute rates in the treated rats were highly significantly less than those of the pair-fed rats ($P 0.001$). Differences in fractional rates were less marked but still significant ($P 0.002$).

(b) Albumin catabolic rate. The absolute catabolic rate fell slowly throughout the study period, becoming significantly less than normal from day 7 onwards ($U 8 < 19$). There was no early post-operative rise. The fractional catabolic rate showed a highly significant post-operative rise ($U 17 < 19$). It then fell, and from day 8 was significantly less than normal ($U 29 < 31$) although still within the normal range. The absolute and fractional catabolic rates were not significantly different from untreated hypophysectomized rats on day 2 (absolute catabolic rate $U 43 > 14$, fractional catabolic rate $U 29 > 14$). On day 9 the absolute ($U 3 < 7$) but not the fractional ($U 17 > 14$) catabolic rate was highly significantly greater in the treated rats. The absolute and fractional

catabolic rates on day 2 ($U 0 < 5$) and absolute catabolic rate on day 9 ($U 1 = 1$) were highly significantly less than the pair-fed group, but there was no significant difference in fractional catabolic rate on day 9 ($U 12 > 5$).

(c) Albumin concentration. The albumin concentration fell sharply initially and then rose to normal during the last 4 days of the study period.

DISCUSSION

Results of hypophysectomy experiments. At all times after hypophysectomy synthesis rates fell far below the normal range. However, since the values on day 2 were not significantly different from those of the pair-fed group of rats, the initial fall in synthesis rate can be accounted for on the basis of a decreased food intake alone. Only later in the study on day 9, when the synthesis rates in the hypophysectomized group were highly significantly less than those of the pair-fed group, did the effects of pituitary insufficiency become demonstrable.

The catabolic rate was also shown to fall after hypophysectomy although this decrease tended to occur 3–4 days after the decrease in synthesis rate. In the light of the known ability of glucocorticoid and thyroid hormones to stimulate albumin catabolism (Cuthbertson, 1964) it becomes possible to

argue that the fall in catabolism was due to a decrease in the amounts of these hormones after hypophysectomy. However, since complete disappearance of plasma corticosterone had occurred some 3–4 days before the fall in catabolism, this argument does not hold in respect of glucocorticoids. Tests of thyroid function were not undertaken. The delayed fall in catabolism may be more satisfactorily accounted for as follows. It is generally accepted that albumin catabolism proceeds as a first-order process geared to the size of the intravascular albumin pool (McFarlane, 1964) and further that the decrease in catabolic rate that occurs after decrease of this pool is an adaptive response aimed at preserving its size (Hoffenberg, Black & Brock, 1966). Since hypophysectomy resulted in an early decrease of albumin synthesis rate and catabolism continued to proceed at normal rates, the net effect was to result in a contraction of the intravascular albumin pool, as was reflected by the falling plasma concentration. Once the pool size had fallen to some critical value, the adaptive fall in catabolism became manifest.

Result of pair-feeding experiments. These experiments confirm the well-recognized decrease and rise in albumin synthesis rate in the face of protein deprivation and repletion respectively (Kirsch *et al.* 1968; Rothschild *et al.* 1968). It is worthy of note that whereas the improved dietary intake on day 9 resulted in increased rates of synthesis in the sham-operated group, pituitary hormonal deficiency seems to have prevented such a response in the hypophysectomized animals.

The catabolic rate apart from an initial early rise remained within normal limits, thus implying that the intravascular albumin pool had not been compromised by decreased synthesis rates to the extent where the compensatory decrease in catabolism would be required. The post-operative rise in albumin catabolism that naturally occurs in association with operative techniques was far greater in the sham-operated group than the hypophysectomized group, and reflects the integral part played by the pituitary gland in the genesis of this response (Cuthbertson, 1964).

Results of growth hormone replacement experiments. Throughout the study period fractional synthesis rates in the growth hormone-treated rats were significantly higher than those of the untreated rats, the latter shown by immunoassay to be growth hormone-deficient. However, the values obtained were still significantly less than normal. Since growth hormone had no significant effect on the post-hypophysectomy anorexia, the best effect growth hormone could be expected to achieve would be to increase the synthesis rate to the value of the pair-fed rats. This effect was achieved on day 2 only. On day 9 the synthesis rate was significantly

lower in the growth hormone group compared with the sham-operated rats. Clearly this low synthesis rate, in the face of both an adequate food intake and growth hormone substitution, implies the participation of other pituitary hormones in the regulation of the synthetic process. In this respect glucocorticoid (Korner, 1960; Bancroft, Levine & Tashjian, 1969) and thyroid hormones (Tata & Widnell, 1966) appear to be of importance.

Late changes in albumin catabolic rate were less pronounced than those shown by untreated hypophysectomized rats, probably as a consequence of the maintenance of albumin synthesis and hence the intravascular pool after growth hormone therapy. This would delay the compensatory decrease in catabolism as has been discussed above.

There is considerable difference of opinion as to the role played by growth hormone in regulating albumin metabolism. Indirect methods of measurement by means of plasma specific radioactivity curves led to the conclusion that growth hormone achieved its effect by stimulating albumin synthesis (Ulrich *et al.* 1954) or by slowing catabolism (Jeejeebhoy *et al.* 1965; Gabuzda *et al.* 1963). However, Jeejeebhoy *et al.* (1970) were unable to observe any effect of growth hormone when synthesis was measured directly in normal rats.

In the present study, treatment with growth hormone is shown to increase the rate of both albumin synthesis and catabolism when compared with hypophysectomized rats. It seems unlikely that growth hormone could regulate albumin concentrations effectively if under its influence both synthesis and catabolism were raised. More probably, the primary action of growth hormone is on albumin synthesis resulting in an increased intravascular pool, the size of which will in turn dictate the rate at which catabolism is to proceed. This effect is likely to be direct as Jefferson & Korner (1967) have shown this hormone to stimulate protein anabolism in the perfused liver. The participation of the anabolic action of insulin is not excluded, however, as insulin is released after growth hormone administration (Frohman, MacGillivray & Aceto, 1967) and has long been considered by Young (1953) to be the major mechanism by which growth hormone achieves its protein anabolic effects.

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REFERENCES

- Bancroft, F. C., Levine, L. & Tashjian, A. H. (1969). *Biochem. biophys. Res. Commun.* **37**, 1028.
- Cuthbertson, D. P. (1964). In *Mammalian Protein Metabolism*, vol. 2, p. 373. Ed. by Munro, H. N. & Allison, J. B. New York: Academic Press Inc.
- Enerbäck, L., Lundin, P. M. & Mellgren, J. (1959). *Acta endocr., Copnh.*, **32**, 552.
- Fernandez, A., Sobel, C. & Goldenberg, H. (1966). *Clin. Chem.* **12**, 194.
- Frohman, L. A., MacGillivray, M. H. & Aceto, T. (1967). *J. clin. Endocr.* **27**, 561.
- Gabuzda, T. G., Jick, H. & Chalmers, T. C. (1963). *Metabolism*, **12**, 1.
- Hoffenberg, R., Black, E. & Brock, J. F. (1966). *J. clin. Invest.* **45**, 143.
- Hunter, W. M. & Greenwood, F. C. (1962). *Nature, Lond.*, **194**, 495.
- Jeejeebhoy, K. N., Boucher, B. J. & Hartog, M. (1965). *Metabolism*, **14**, 67.
- Jeejeebhoy, K. N., Bruce-Robertson, A., Sodtke, U. & Foley, M. (1970). *Biochem. J.* **119**, 243.
- Jefferson, L. S. & Korner, A. (1967). *Biochem. J.* **104**, 826.
- Jones, E. A., Craigie, A., Tavill, A. S., Simon, W. & Rosenoer, V. M. (1968). *Clin. Sci.* **35**, 553.
- Kirsch, R., Frith, L., Black, E. & Hoffenberg, R. (1968). *Nature, Lond.*, **217**, 578.
- Koj, A. & McFarlane, A. S. (1968). *Biochem. J.* **108**, 137.
- Korner, A. (1960). *J. Endocr.* **21**, 177.
- Korner, A. & Debro, J. R. (1956). *Nature, Lond.*, **178**, 1067.
- Lee, M. & Ayres, G. B. (1936). *Endocrinology*, **20**, 489.
- Levin, L. (1944). *Am. J. Physiol.* **141**, 143.
- Levin, L. & Leatham, J. H. (1942). *Am. J. Physiol.* **136**, 306.
- Li, C. H. & Reinhardt, W. O. (1947). *J. biol. Chem.* **167**, 487.
- McFarlane, A. S. (1958). *Nature, Lond.*, **182**, 53.
- McFarlane, A. S. (1963). *Biochem. J.* **89**, 277.
- McFarlane, A. S. (1964). In *Mammalian Protein Metabolism*, vol. 1, p. 331. Ed. by Munro, H. N. & Allison, J. B. New York: Academic Press Inc.
- McFarlane, A. S., Irons, L., Koj, A. & Regoecki, E. (1965). *Biochem. J.* **95**, 536.
- Marsh, W. H., Fingerhut, B. & Kirsch, E. (1957). *Techn. Bull. Regist. med. Technol.* **27**, 239.
- Matthews, C. M. E. (1961). *J. clin. Invest.* **40**, 603.
- Mattingly, D. (1962). *J. clin. Path.* **15**, 374.
- Reeve, E. B., Pearson, J. R. & Martz, D. C. (1963). *Science, N.Y.*, **139**, 914.
- Rothschild, M. A., Oratz, M., Mongelli, J. & Schreiber, S. S. (1968). *J. clin. Invest.* **47**, 2591.
- Sellers, A. L., Bonorris, G. & Katz, J. (1969). In *Physiology and Pathophysiology of Plasma Protein Metabolism*, p. 95. Ed. by Birke, G., Norberg, R. & Plantin, L. O. Braunschweig: Pergamon Press.
- Siegel, S. (1956). *Nonparametric Statistics for the Behavioural Sciences*. Tokyo: McGraw-Hill Book Co. Inc.
- Tata, J. R. & Widnell, C. C. (1966). *Biochem. J.* **98**, 604.
- Tavill, A. S., Craigie, A. & Rosenoer, V. M. (1968). *Clin. Sci.* **34**, 1.
- Ulrich, F., Tarver, H. & Li, C. H. (1954). *J. biol. Chem.* **209**, 117.
- Yalow, R. S. & Berson, S. A. (1960). *J. clin. Invest.* **39**, 1157.
- Young, F. G. (1953). *Recent Prog. Horm. Res.* **8**, 471.