Vol. XLIX, No. 3 (June, 1968) was issued on 1.7.68.

THE BRITISH JOURNAL OF
EXPERIMENTAL PATHOLOGY

VOL. XLIX AUGUST, 1968 NO. 4

THE REMOVAL OF INJECTED BERYLLIUM FROM THE BLOOD OF THE RAT

THE ROLE OF THE RETICULO-ENDOTHELIAL SYSTEM

J. VACHER AND H. B. STONER

From the Toxicology Research Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey

Received for publication January 3, 1968

THE distribution, and hence the toxic effects of a soluble salt of beryllium injected intravenously depends on the actual dose given and on the forms in which it is transported in the blood. The smaller the amount injected, the greater the fraction excreted in the urine or taken up by bone, so that the injection of carrier-free ^{7}Be does not reproduce the distribution of toxic doses of beryllium (Scott, Neuman and Allen, 1950). After the injection of $BeSO_4$ the beryllium in the blood can be divided into a diffusible fraction, associated with plasma organic acids such as citrate, and a non-diffusible fraction (Feldman, Havill and Neuman, 1953). In a previous paper (Vacher and Stoner, 1968) we extended the work of others (Feldman *et al.*, 1953; Cheng, 1956; Belman, 1957; Reeves and Vorwald, 1961) on the non-diffusible fraction and showed that when $BeSO_4$ is injected i.v. beryllium phosphate is produced in the plasma and that most, if not all, of the aggregates formed are bound to plasma globulin, most probably α-globulin.

The main lesion produced in the rat by toxic i.v. doses of $BeSO_4$ is " mid-zonal " necrosis of the liver (Scott, 1948; Aldridge, Barnes and Denz, 1949). Although it is not yet known how beryllium produces its toxic effects within the cells (Aldridge, 1966), a considerable step towards explaining the site of this lesion was made by Cheng (1956). He proposed that the colloidal beryllium phosphate in the blood was ingested by the Kupffer cells of the liver and that beryllium first damaged these cells and later diffused into the adjacent parenchymal cells causing their necrosis. The localization of the necrosis to the " mid-zone " of the classical liver lobule could then be explained by the concentration of Kupffer cells in the afferent terminal vessels in this area (Rappaport, Borowy, Lougheed and Lotto, 1954).

$BeSO_4$ has also been used to depress the activity of the reticulo-endothelial (RE) system (Paget, 1961; Stoner, 1961) and could have a wider application in the study of phagocytosis by cells of this system. The formation of particles within the circulation, as after the injection of $BeSO_4$, creates a rather different situation from that produced by the injection of actual particles, such as those of carbon or thorium dioxide, which are always given with suspending materials such as gelatin or dextrin. These suspending agents are not neutral in the reactions concerned with the removal of the particles (Normann and Benditt, 1965; Koenig, Heyssel, Melly and Rogers, 1965). Using $BeSO_4$ the influence of these suspending agents could be avoided. The removal of beryllium from the circulation has therefore been studied from this point of view.

MATERIALS AND METHODS

Female albino rats of the Porton strain weighing between 190–220 g. and fed on MRC diet 41B. (Bruce and Parkes, 1956) were used. Doses of $BeSO_4$ (AnalaR, B.D.H.) are expressed in terms of Be. The $BeSO_4$ solution was made radioactive by the addition of $^7BeCl_2$ (Radiochemical Centre, Amersham) to give a mixture of known specific activity.

For experiments on the rate of removal of circulating beryllium the left jugular vein was cannulated with polythene tubing (internal diam. 0·4 mm.) under ether anaesthesia 2–5 days before the experiments (Ginsburg and Heller, 1953). On the day of the experiment the rats were given 75 i.u. heparin per 100 g. body wt. via the cannula, 10 min. to 2 hr. before the dose of labelled $BeSO_4$ was given by the same route in a volume of 0·1 or 0·2 ml. per 100 g. body weight. This difference in volume size did not affect the results. The injection time was 10 sec. ^7Be was not adsorbed by the polythene of the cannula.

To determine the rate of clearance of beryllium from the circulation, samples of blood (approx. 0·05 ml.) were withdrawn from the cannula, at intervals after the injection, by attaching another polythene tube to it and sucking up a small amount of blood. This blood was discharged into a small tared test tube, weighed and its radioactivity measured. For convenience the results are expressed per g. blood. The blood in the dead space of the cannula was removed before each sample. Clearance rates were calculated according to the formula:

$$k = \frac{\log C_1 - \log C_2}{t_2 - t_1} \times 2{\cdot}303$$

where C_1 and C_2 are the concentrations of beryllium in the blood at times t_1 and t_2.

In some experiments, the animals were killed 2·5 hr. after injection, and tissues removed for assay. The spleen, lungs and kidneys were examined whole. Samples were taken from the abdominal muscles, femur and from each lobe of the liver. The results have been expressed as the percentage of the dose of beryllium recovered in the organs, assuming that the musculature accounted for 50 per cent and the skeleton 6 per cent of the body wt. (Caster, Poncelet, Simon and Armstrong, 1956).

The radioactivity of the blood and tissue samples was measured with a Packard Autogamma scintillation spectrometer.

RESULTS

The i.v. LD_{50} of $BeSO_4$ in these female rats was 0·51 mg. Be per kg. body wt. (Vacher and Stoner, 1968). As the experiments reported here were completed within 3 hr. of the injection, no gross toxic effects of the beryllium were observed.

Removal of beryllium from blood

Carrier-free ^7Be disappeared very rapidly from the circulation after intravenous injection. A typical curve showing the early rapid and later more gradual fall in log. concentration is shown in Fig. 1. The disappearance of beryllium from the circulating blood after the i.v. injection of different doses of $BeSO_4$ is illustrated

in Fig. 2, each curve showing the results in a single rat. With doses of beryllium > 0·03 mg. per kg. body wt. there was little variation between the curves obtained from different rats given the same dose. These curves show the changes from 3 min. after the injection. The concentration at zero time (C_0) was calculated on the assumption of instantaneous mixing in a blood volume of 7 per cent of the body wt. Separate experiments showed that during these first few min. the concentration of beryllium in the blood fell very rapidly. For the remaining 2·5 hr. of the experiment the concentration fell much more slowly in an exponential fashion. Extrapolation of this second part of the curve back to zero time did not pass through C_0 but through a much lower concentration C'_0. These curves were much more strikingly biphasic than those for carrier-free ^{7}Be.

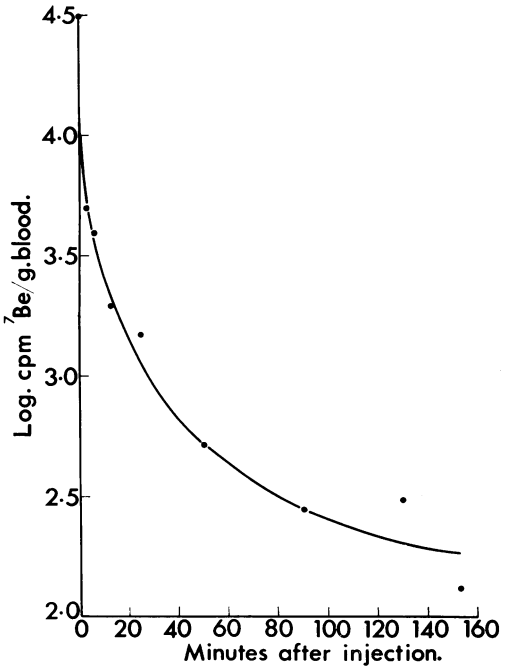

FIG. 1.—Disappearance of radioactivity from the blood stream of a rat after the i.v. injection of carrier-free $^7BeCl_2$.

Further information about the removal of beryllium from the blood was derived from a more detailed examination of these curves. The relationships between C'_0 and $(C_0 - C'_0)$ and dose are shown in Fig. 3. The change in the latter relationship when the dose exceeded 0·13 mg. Be per kg. body wt. implies that more than one process is concerned in the early removal of the higher doses of beryllium. The amount removed by the mechanisms involved in the first stage could be calculated from $(C_0 - C'_0)$ and was always a substantial fraction of the total amount removed in 2·5 hr. This fraction was greatest at the extremes of the dose range, 0·82 for 0·03 mg. and 0·64 for 0·51 mg. per kg. body wt. The average values for the intermediate doses varied between 0·29 and 0·46.

The clearance rates for the second part of the curves (3 — 150 min.) fell in a regular fashion as the dose increased. The relationship between the rate (k)

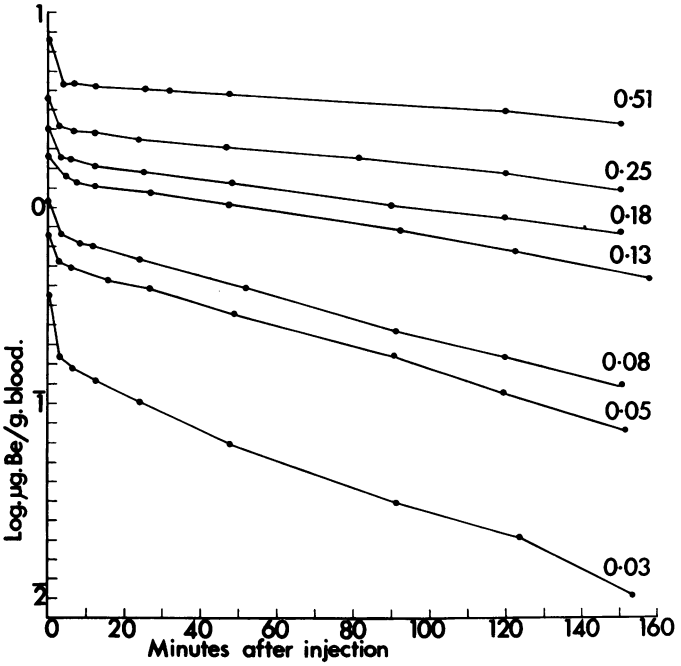

FIG. 2.—Representative curves showing the disappearance of beryllium from the blood stream of the rat after the i.v. injection of different doses of $BeSO_4$. Each curve represents a single rat. The doses (mg. Be per kg. body wt.) are indicated by the figures attached to each curve.

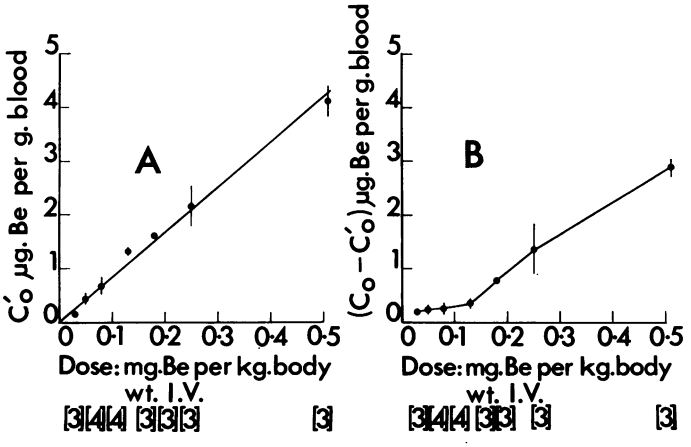

FIG. 3.—The relationship (A) between C'_0 and (B) between $(C_0 - C'_0)$ and the i.v. dose of $BeSO_4$. For definitions of C_0 and C'_0 see text. The points represent mean values and the standard deviations are shown by vertical lines when they exceed the size of the points. The number of rats given each dose is shown in parentheses. Curve for C'_0 fitted by eye.

and the initial concentration of beryllium in the blood for this part of the curve (C'_0) is shown in Fig. 4. An initial rate of removal of beryllium from the blood stream by the mechanism responsible for this part of the curve could be calculated from k and C'_0 for each of the doses used and its relationship to C'_0 is shown in Fig. 5. With increasing doses, giving higher initial blood concentrations, this rate increased at first quickly but later more slowly.

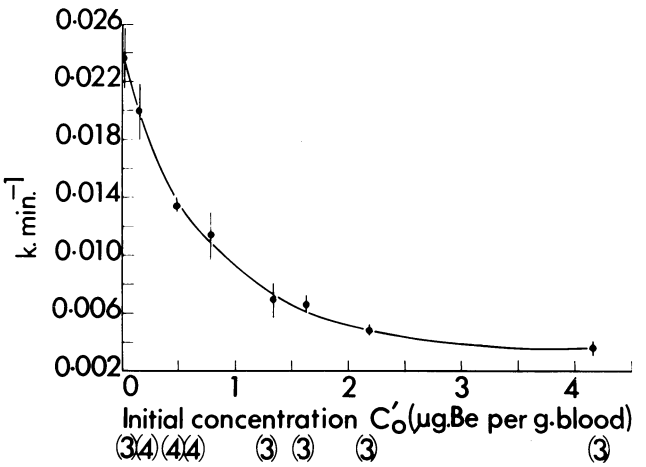

FIG. 4.—The relationship between the rate of removal of Be from the blood (k) and the initial concentration of Be in the blood (C'_0). For definition of C'_0 see text. The points represent the means of the values obtained in the number of rats shown in parentheses and the standard deviations are shown by the vertical lines. The values at $C'_0 = 0$ were obtained with carrier-free ^{7}Be. Curve fitted by eye.

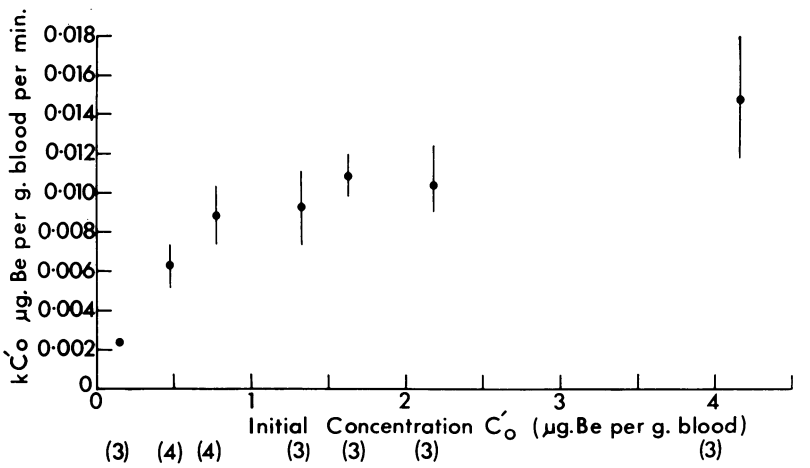

FIG. 5.—The relationship between the initial rate of removal of Be from the blood (k C'_0) and the initial concentration of Be in the blood (C'_0). For definition of C'_0 see text. The points represent the means of the values obtained in the number of rats shown in parentheses and the standard deviations are shown by the vertical lines.

Tissue distribution

The distribution of beryllium in the tissues 2·5 hr. after its intravenous injection as $BeSO_4$ is shown in Table I. Between 40–50 per cent of the dose was recovered in the tissues studied. For any particular dose, uptake by the lungs was most variable. The fraction taken up by the skeleton and kidneys was inversely proportional to the dose. In the liver and spleen the percentage uptake was independent of the amount given for doses > 0·05 mg. Be per kg. body wt. All the rats in Table I had in-dwelling jugular cannulae but not all had had repeated blood sampling before being killed. Comparison of the separated groups showed that the repeated removal of small samples of blood to determine the clearance rate did not affect the distribution of beryllium and all the results were pooled for Table I.

TABLE I.—*The Distribution of Beryllium 2·5 hr. After its i.v. Injection as $BeSO_4$ or as Carrier-free ^7Be.*

Dose mg. Be per kg. body weight	Liver	Spleen	Lungs	Kidneys	Musculature	Skeleton
	Percentage of dose (Mean ± S.E.)					
0·51 (17)	18·1±1·2	2·0±0·1	3·5±0·4	1·1±0·1	8·8±0·7	9·6±0·8
0·25 (6)	19·8±3·4	2·1±0·2	5·0±2·1	0·9±0·1	5·6±1·4	7·3±0·7
0·18 (3)	14·8±0·5	1·6±0·1	13·0±3·4	1·1±0·1	5·8±0·9	11·7±2·0
0·13 (5)	19·9±2·8	2·2±0·4	7·7±3·2	1·4±0·1	5·4±0·7	11·0±1·3
0·08 (7)	22·8±3·8	1·9±0·5	3·6±1·0	1·9±0·2	3·8±0·4	14·8±2·2
0·05 (4)	13·4±1·7	1·1±0·2	2·1±0·7	2·4±0·3	4·0±0·9	24·0±2·5
0·03 (4)	6·5±1·2	0·3±0·1	0·6±0·3	4·6±1·1	3·0±1·1	29·7 (24·7, 34·6)
^7Be (3)	5·7±1·3	0·1±0·03	0·2±0·03	2·6±0·19	1·1±0·2	30·2 (31·3, 29·0)

No. of rats shown in parentheses. Where there were only 2 samples available the individual values are given in parentheses after the mean.

DISCUSSION

These findings must be considered in the light of the chemical events occurring in the plasma after the i.v. injection of $BeSO_4$ (Feldman *et al.*, 1953; Vacher and Stoner, 1968). The state of beryllium in plasma depends on the relative proportions of beryllium, phosphate and organic acids present. The change in the pH of the acid injection fluid as it mixes with the plasma will also be important. Citric is the most abundant of the organic acids concerned but, *in vivo*, 1 mole would probably not complex more than 1 mole of beryllium (Feldman, Toribara, Havill and Neuman, 1955). Assuming a citrate concentration of 2·5 mg. per 100 ml. plasma (Krebs, 1950) the maximum dose of Be which could be complexed in this way would be about 0·04 mg. per kg. body wt., if it mixed instantly with all the plasma. This will not be achieved in practice. Some beryllium phosphate is probably always formed. As the dose of $BeSO_4$ is increased more phosphate will be formed and bound by plasma protein (α-globulin). Although most of the beryllium phosphate aggregates in rat plasma are globulin bound a few may remain free (Vacher and Stoner, 1968).

In common with others (Scott *et al.*, 1950; Klemperer, Martin and Liddy, 1952) we found an inverse relationship between the dose and the percentage uptake by kidney and bone. For kidney and, to a less extent, for bone this can be explained by the decreasing percentage of the dose which remains diffusible as the dose of

$BeSO_4$ is increased. Part of the bone content must also be due to the uptake of non-diffusible aggregates by the RE cells of the marrow or to the trapping of aggregates in its sinuses.

The difference between the shapes of the disappearance curves in Figs. 1 and 2 can be tentatively explained as follows. For carrier-free ^7Be the disappearance of the diffusible fraction was the most important event but with the addition of carrier the contribution from this fraction diminished and the removal of beryllium phosphate aggregates became more important. Similar biphasic clearance curves have been found in mice injected i.v. with radioactive $BeSO_4$ (Vacher, unpublished). Since the radioactive and non-radioactive parts of the dose were both injected in solution the difficulty of radioactive tracers discussed by Scott, Williams and Marriott (1967) is avoided.

The RE system is primarily responsible for removing these aggregates from the circulation although the ultimate distribution of the beryllium will extend beyond this system (Cheng, 1956; Witschi and Aldridge, 1968). The work of Dobson (1957) on the removal of particles from the circulation by the RES suggests that the biphasic curves in Fig. 2 could be explained by the formation of different sized aggregates of beryllium phosphate-α-globulin complex after the i.v. injection of $BeSO_4$. The large ones would be removed rapidly and the small ones more slowly. The events of the first few minutes are clearly complicated *e.g.* Fig. 3B and the variations in the fraction of the dose removed in the first 3 min. (see above). These events do not seem to have been observed previously through failure to take early samples in experiments with ^7Be + carrier. It is unlikely that the whole of this effect, at all doses, can be explained in terms of particle size, although this may be the most important factor for the higher doses. These complexities limit the usefulness of labelled $BeSO_4$ as a tracer for studying the kinetics of the RES and the data could not be easily fitted to a model of the type proposed by Gabrieli and Snell (1965).

Once the events of the first few min. are completed the clearance of the blood followed a more regular pattern, representing the removal of small particles whose initial concentration (C'_0) was proportional to the dose of $BeSO_4$ (Fig. 3A). The particular interest of this part of the experiments lies in the relationship between the rate of the removal of these particles and their initial concentration in the blood shown in Fig. 4. In this respect the results resemble those obtained with other colloid particles (Biozzi, Benacerraf and Halpern, 1953; Benacerraf, Biozzi, Halpern and Stiffel, 1957; Dobson, 1957) but are particularly interesting because the particles were generated within the plasma and no foreign suspending agents were used (Normann and Benditt, 1965; Koenig *et al.*, 1965). This relationship seems to be an inherent property of the RES.

The larger aggregates of the beryllium phosphate-α-globulin complex would be expected to localize in the liver and spleen (Dobson, 1957). Comparisons with other data obtained in these laboratories (Witschi and Aldridge, 1968) showed that at 2·5 hr. uptake by these organs was about half that at 24 hr. The fraction of the dose removed by the liver and spleen was small in comparison with that for other commonly used particles such as carbon (Benacerraf *et al.*, 1957) but there was no evidence for the saturation of this pathway *i.e.* no fall in the percentage of the dose found in these organs as the dose was increased. Witschi and Aldridge (1968) used doses up to 0·99 mg. Be per kg. body wt. and only then was there any tendency for the percentage hepatic uptake in 24 hr. to fall. It is

not yet possible to explain the variation in the initial rate, kC'_0, with dose because of the number of possible factors involved.

These results support the view that the inhibition of carbon clearance found previously in rats given small doses of $BeSO_4$ (Stoner, 1961) was due to a toxic effect on the Kupffer cells. Histological evidence of damage to these cells has been observed by Cheng (1956) and by ourselves (unpublished). Indeed, this effect, which will inhibit the uptake of further doses of beryllium (Vacher, unpublished), will limit the amount of injected $BeSO_4$ which can be removed by the RES. While $BeSO_4$ has no advantages as a tracer for studying the function of the RES its ability to depress the activity of the Kupffer cells by its toxic effect at doses which do not seriously impair the function of the parenchymal cells nor " saturate " the Kupffer cells could make it a useful tool for the further investigation of this system.

SUMMARY

The disappearance from the circulation of beryllium injected either as carrier-free ^7Be or as $BeSO_4$ labelled with ^7Be has been studied in rats and the findings interpreted in the light of previous work on the transport of beryllium in blood.

Beryllium disappeared much more slowly from the circulation when injected as $BeSO_4$ instead of as carrier-free ^7Be. This was because only a small part of the dose of $BeSO_4$ remained in a diffusible form, most of it being converted to a beryllium phosphate-α-globulin complex which was removed by the reticulo-endothelial system.

The disappearance curves for $BeSO_4$ were biphasic. During the second phase of the removal of beryllium an inverse relationship between dose and rate of removal was found, as for other more commonly used particles. This was of particular interest as these beryllium particles were generated within the plasma and no foreign suspending agents were used.

With the doses used there was no evidence of saturation in the liver or spleen and the inhibition of carbon clearance found in previous experiments after small doses of $BeSO_4$, was attributed to a toxic effect on the cells of the reticulo-endothelial system.

Our thanks are due to Miss J. Lumbers for her skilful and valuable technical assistance.

REFERENCES

ALDRIDGE, W. N.—(1966) *Lab. Invest.*, **15**, 176.

ALDRIDGE, W. N., BARNES, J. M. AND DENZ, F. A.—(1949) *Br. J. exp. Path.*, **30**, 375.

BELMAN, S.—(1957) Ph.D. Thesis. New York University.

BENACERRAF, B., BIOZZI, G., HALPERN, B. N. AND STIFFEL, C.—(1957) *In* ' Physiopathology of the Recticulo-endothelial System '. Eds. B. N. Halpern, B. Benacerraf and J. F. Delafresnaye. Oxford (Blackwell), p. 52.

BIOZZI, G., BENACERRAF, B. AND HALPERN, B. N.—(1953) *Br. J. exp. Path.*, **34**, 441.

BRUCE, H. M. AND PARKES, A. S.—(1956) *J. Anim. Tech. Ass.*, **7**, 54.

CASTER, W. O., PONCELET, J., SIMON, A. B. AND ARMSTRONG, W. D.—(1956). *Proc. Soc. exp. biol. med.*, **91**, 122.

CHENG, K. K.—(1956) *J. Path. Bact.*, **71**, 265.

DOBSON, E. L.—(1957) *In* ' Physiopathology of the Reticulo-endothelial System '. Eds. B. N. Halpern, B. Benacerraf and J. F. Delafresnaye, Oxford (Blackwell), p. 80.

FELDMAN, I., HAVILL, J. R. AND NEUMAN, W. F.—(1953) *Archs. Biochem.*, **46**, 443.
FELDMAN, I. TORIBARA, T. Y., HAVILL, J. R. AND NEUMAN, W. F.—(1955) *J. Am. Chem. Soc.*, **77**, 878.
GABRIELI, E. R. AND SNELL, F. M.—(1965) *J. reticuloendothelial Soc.*, **2**, 141.
GINSBURG, M. AND HELLER, H.—(1953) *J. Endocrin*, **9**, 267.
KLEMPERER, F. W., MARTIN, A. P. AND LIDDY, R. E.—(1952) *Archs. Biochem.*, **41**, 148.
KOENIG, M. G., HEYSSEL, R. M., MELLY, M. A. AND ROGERS, D. E.—(1965) *J. exp. Med.*, **122**, 117.
KREBS, H. A.—(1950) *Ann. Rev. Biochem.*, **19**, 409.
NORMANN, S. J. AND BENDITT, E. P.—(1965) *J. exp. Med.*, **122**, 693.
PAGET, G. E.—(1961) *Toxicol. appl. Pharmacol.*, **3**, 595.
RAPPAPORT, A. M., BOROWY, Z. J., LOUGHEED, W. M. AND LOTTO, W. N.—(1954) *Anat. Rec.*, **119**, 11.
REEVES, A. L. AND VORWALD, A. J.—(1961) *J. occup. Med.*, **3**, 567.
SCOTT, G. B. D., WILLIAMS, H. S. AND MARRIOTT, P. M.—(1967) *Br. J. exp. Path.*, **48**, 411.
SCOTT, J. K.—(1948) *Archs. Path.*, **45**, 354.
SCOTT, J. K., NEUMAN, W. F. AND ALLEN, R.—(1950) *J. biol. Chem.*, **182**, 291.
STONER, H. B.—(1961) *Br. J. exp. Path.*, **42**, 523.
VACHER, J. AND STONER, H. B. (1968) *Biochem. Pharmac.*, **17**, 93.
WITSCHI, H. P. AND ALDRIDGE, W. N.—(1968) *Biochem. J.*, **106**, 81.