Summary

Attention is drawn to the conflicting results reported by investigators on the effect of sarcoidosis and other diseases of the reticulo-endothelial system on circulating antibody production. Since previous studies were concerned chiefly with the response to reimmunization, we decided to test the response to this and to primary inoculation, using tetanus toxoid as the antigen in both instances.

Twelve patients with sarcoidosis produced significantly less circulating antitoxin after primary immunization than did 14 controls. The poorest responders were those in whom the sarcoid process was "active."

A normal response to reimmunization was given by 10 patients with sarcoidosis who had been immunized against tetanus some years earlier.

The serum staphylococcal alpha-antitoxin level of 50 patients with sarcoidosis and 19 patients with reticulosis was of the same order as that of 75 control patients.

The study of a small number of patients with reticulosis suggested that a poor response to primary immunization also occurs in this condition.

We are indebted to Dr. Clifford Hoyle for his advice and encouragement in the preparation of this paper; our thanks are due to him, and to Dr. A. Gilpin and Dr. E. W. H. Shawcross for permission to investigate patients under their care. Mr. P. A. Young, of the Wellcome Research Laboratories, kindly carried out the statistical analysis of the data in Table I.

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Nearly one million Arab refugees from Palestine are now living in the Gaza Strip, Jordan, Lebanon, and Syria. Now, nearly ten years after their exodus from their homes, they remain mainly dependent upon the United Nations Relief and Works Agency for Palestine Refugees (U.N.R.W.A.) for their basic needs. Besides providing them with food, shelter, welfare services, and education, U.N.R.W.A. operate a large and comprehensive health service, the technical direction of which remains the responsibility of the World Health Organization. The unsatisfactory living conditions of the refugees in an area like the Middle East which is especially open to epidemics have prompted U.N.R.W.A. to give special emphasis to preventive measures in its overall health programme. Under the supervision of a sanitary engineer seconded by W.H.O., strict sanitation measures are applied in all camps. These include the provision of a clean water supply, an efficient garbage disposal system, and proper sewage arrangements. Flies and other insects which help in spreading disease are combated through regular spraying with insecticides. Health education workers are now employed in camps teaching refugees the elements of hygiene and how to combat the spread of disease through proper health habits. Malaria is now on the way to being eradicated. Last year 254,000 refugees were inoculated against typhoid, 497,000 against smallpox, 51,000 against diphtheria, and 32,000 against whooping-cough.

METABOLISM OF ¹³¹I-LABELLED
ALBUMIN IN AFRICAN SUBJECTS

BY

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Reports from Africa (Arens and Brock, 1954; Holmes *et al.*, 1955), Jamaica (Garrow, 1954), and the United States of America (Milam, 1946; Rawnsley *et al.*, 1956) have consistently shown that Negroids have lower serum albumin levels than Caucasoids living in the same regions. In this investigation an attempt has been made to establish whether the two racial groups also differ in regard to albumin distribution and turnover. ¹³¹I-labelled albumin was injected into five healthy African male subjects, and rates of albumin katabolism were calculated from plasma specific activity curves as well as from urinary ¹³¹I-excretion data. Results are compared with those obtained by McFarlane and co-workers (personal communication), who have studied the turnover of the same labelled albumin in a group of young English adult males.

Validity of the ¹³¹I-albumin Label

The validity of metabolic data obtained through the use of ¹³¹I-labelled plasma proteins has been seriously questioned in recent years (Goldsworthy and Volwiler, 1957). Published reports indicate that the metabolic fate of ¹³¹I-labelled molecules is greatly influenced by such factors as the method of preliminary protein fractionation (McFarlane, 1956), pasteurization of the protein solution (Merchant *et al.*, 1957), the degree and evenness of iodination (McFarlane, 1956), and the occurrence of radiation damage to the protein prior to injection (Yalow and Berson, 1957). The studies of McFarlane and his co-workers have shown, however, that the *in vivo* behaviour of their ¹³¹I-labelled albumin and globulin in both the rabbit (Cohen *et al.*, 1956) and the rat (Campbell *et al.*, 1956) is almost identical with that of the corresponding proteins labelled with ¹⁴C by the biosynthetic procedure. Moreover, the fate of ¹⁴C and ¹³¹I-labelled antibody globulins is identical in either the presence or absence of an immune response (McFarlane, 1957).

Since it is generally accepted that protein molecules are not significantly altered by substitution of ¹⁴C for natural carbon, these experiments show that under certain conditions of fractionation and iodination ¹³¹I can serve as a reliable protein label in biological investigations. In addition to its simplicity, the method has the advantage that the ¹³¹I released during protein breakdown is quantitatively excreted, and this provides a valuable independent measure of the rate of katabolism.

Methods

Preparation of Albumin for Iodination.—Albumin was prepared from the serum of healthy European subjects by two different methods. The first preparation was made according to the ether fractionation procedure of Kekwick and Mackay (1954) and was incubated at 60° C. for 12 hours prior to iodination. Albumin was also prepared by zone electrophoresis on a column of acetylated cellulose (Porath, 1954).

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Iodination.—Albumin solutions were labelled with ^{131}I according to the procedure described by McFarlane (1956). About 25% of the radioactivity used was bound to protein, and this corresponded to 1.03 atoms of iodine per molecule of albumin (molecular weight 65,000). Less than 2% of the total radioactivity was present in the supernatant after precipitation with 10% trichloroacetic acid. The albumin solution was Seitz-filtered in the presence of 1 ml. of normal human serum, which reduces absorption losses on the filter and minimizes radiation damage during

storage. The labelled protein was injected six to eight days after iodination, at which time the maximum radiation dosage was 2.6 kilorads, which is well below the level apt to cause radiation damage to albumin solutions. Analysis of the iodinated albumin solutions by paper electrophoresis and strip counting showed that radioactivity was confined to the albumin peak (Fig. 1).

Subjects.—The five subjects investigated were healthy African males between 18 and 45 years of age; all had received an adequate balanced hospital diet for at least five months prior to these tests. Four were ward orderlies who had been in the employ of the hospital for at least one year. The other (Table I, subject B) was a labourer who had been admitted with tuberculous meningitis five months previously; at the time of this experiment he had been afebrile for four months and was clinically completely recovered. All subjects received sodium iodide (gr. 10 (0.65 g.) t.d.s.) for two days before the injection of labelled albumin and subsequently throughout the period of blood sampling.

Experimental Procedure.—A measured dose of iodinated albumin for each subject was placed in a rubber-capped container. Practically all of this solution was injected into the antecubital vein. The residual radioactivity retained in the syringe, needle, and bottle was measured and the dose injected (128 to 179 μC) calculated by difference. Blood samples (2 to 4 ml.) were collected into an ammonium and potassium oxalate mixture (Wintrobe, 1946). The first was taken exactly five minutes after injection, and subsequent samples were collected at intervals of one to five days. Measured volumes of plasma were diluted to 3 ml. and counted in a well-type scintillator. The protein content of plasma samples was determined by the biuret method of Gornall *et al.* (1949). The initial plasma samples were analysed by paper electrophoresis and the percentage concentration of albumin was determined by elution of strips (Jencks *et al.*, 1955). The urine passed during 24-hour periods was pooled and the radioactivity of 3-ml. samples measured.

Results

The disappearance curves for labelled albumin in the five subjects studied are shown graphically in Fig. 2. The plasma activity (μC per ml. of plasma) five minutes after injection was taken as 100% and provided a measure of plasma volume. The subsequent plasma activities plotted on a semilogarithmic scale show the usual rapid initial decrease associated with mixing of labelled molecules with extravascular plasma protein; this is followed by an apparently linear rate of decrease. The half-lives measured from the slope of the linear portion were 17 and 17½ days in subjects A and B, who received ether-fractionated albumin, and 20, 21, and 20 days in the remaining subjects injected with electrophoretically separated albumin.

It is often assumed that the half-life provides an accurate measure of metabolic turnover. However, it appears likely that albumin breakdown occurs exclusively within or in close proximity to the intravascular pool (Berson and Yalow, 1954; Campbell *et al.*, 1956). This, as pointed out by McFarlane (1957), implies that albumin specific activities will always be lower in the plasma than in the extravascular compartments after the initial phase of equilibration. Relatively highly labelled molecules will therefore continually enter the plasma from the lymph and produce an apparent lengthening of the

FIG. 1.—Electrophoretic analysis and radioactive assay of ^{131}I -labelled albumins added to normal human serum. The positions of the radioactive peaks show that both labelled protein fractions migrate with albumin on paper electrophoresis.

FIG. 2.—Plasma activity curves in five healthy African subjects. Katabolic rates are obtained from daily urinary ^{131}I excretion and are expressed as a percentage of the intravascular albumin pool broken down daily.

half-life beyond that due to metabolic breakdown alone. Matthews (1957), using a method based on the theory of Rescigno (1956), has shown that the true rate of protein breakdown as well as the relative sizes of intravascular and extravascular pools can be calculated by graphic analysis of plasma activity curves. This method of analysis has given the extravascular to intravascular pool ratios and albumin katabolic rates shown in Table I.

The total intravascular albumin determined from the plasma volume and percentage concentration of albumin was 85 to 102 g. The mass of extravascular albumin was 1.32 to 1.52 times greater than that present in the circulating plasma, so that the total exchangeable albumin was 197 to 257 g. The calculated katabolic rates varied from 8.4 to 10.5% of the intravascular albumin pool per day, which amounts to 7.1 to 10.5 g. per day or 113 to 150 mg. per kg. body weight per day.

The katabolic rate can also be determined directly by measurement of ^{131}I excretion, since the label liberated during protein breakdown is quantitatively excreted in subjects receiving adequate amounts of inactive iodide (Cohen *et al.*, 1956). The katabolic rate is obtained from the daily excretion (24-hour urinary ^{131}I + 5% for faecal loss) expressed as a percentage of the mean intravascular radioactivity during the preceding 24 hours. In subjects A and B, who received ether-fractionated albumin, the katabolic rate was 8 to 17% per day. The rate was consistently higher during the initial 10 to 14 days as compared with the last week of the experiment (Fig. 2). This suggests that a proportion of the labelled molecules were relatively rapidly katabolized, probably as a result of preliminary incubation of the albumin solution (Yalow and Berson, 1957). Katabolic rates in subjects C, D, and E, who received the electrophoretic albumin, were 8 to 12%, and showed no tendency to fall with the progress of the experiment. Mean rates of albumin katabolism derived from urinary excretion data and expressed as a fraction of the intravascular pool are within 2% of the rates calculated from plasma activity curves (Table I).

Comparison of Albumin Turnover in Africans and Europeans

The measurement of albumin distribution and turnover in normal human subjects by means of the ^{131}I -labelling technique has not yielded consistent results in the hands of different investigators. Thus, in normal subjects the absolute rate of albumin turnover has been found to vary from 173 mg. (Schwartz and Thomsen, 1957) to 418 mg.

(Margen and Tarver, 1956) per kg. body weight per day, while mean half-lives of from 10 days (Sterling, 1951) to 23 days (Schwartz and Thomsen, 1957) have been reported. As pointed out above, this wide variation is undoubtedly attributable in large measure to differences in the methods of preliminary albumin fractionation and ^{131}I -labelling. Denatured albumin molecules are rapidly katabolized, so that material giving the longest half-life and smallest rate of turnover probably approximates most closely to the behaviour of native albumin. Nevertheless, it remains difficult to select published results with which the African data can be usefully compared.

A direct comparison can, however, be made between the three Africans who received electrophoretically separated albumin and four young British subjects injected with the same material (T. Freeman, C. M. E. Matthews, and A. S. McFarlane, unpublished observations). Results obtained in the British and African subjects are compared in Table II. From this data it appears that the half-life of labelled albumin is similar in the two groups. The Africans have a low percentage concentration of albumin and a relatively small intravascular pool. Although the ratio of extravascular to intravascular albumin is higher in the Africans, the total exchangeable pool is consistently smaller than in the white subjects. The average rate of albumin katabolism in the African (127 mg. per kg. body weight per day) is somewhat less than in the British subjects (147 mg. per kg. body weight per day). No detailed conclusions can be drawn from these limited data. The results indicate, however, that the relatively low concentration of albumin consistently found in healthy African subjects is not associated with any striking difference in the albumin turnover rate as compared with normal European subjects.

Summary

The distribution and turnover of ^{131}I -labelled albumin have been studied in five healthy male African subjects.

The circulating plasma in these subjects contained 85 to 102 g. of albumin. The extravascular albumin pool was 1.32 to 1.52 times greater than the intravascular pool.

The rate of albumin katabolism calculated from plasma activity curves was 113 to 150 mg. per kg. body weight per day. Katabolic rates estimated from urinary ^{131}I -excretion data were within 2% of those calculated from plasma activity measurements.

TABLE I.—Albumin Distribution and Turnover Data in Five Healthy African Males

Subject	Age	Wt. (kg.)	Total Plasma Protein (g./100 ml.)	Albumin (g./100 ml.)	Plasma Volume (l.)	Intra-vascular Albumin (g.)	Pool Ratio	Total Exchangeable Albumin (g.)	Half-life (days)	Albumin katabolism			
										% per Day (x)	% per Day (y)	g. per Day	mg. per kg. per Day
A	30	88.5	7.7	3.43	2.92	100	1.33	233	17	10.5	12.5	10.5	119
B	26	67.7	7.3	3.07	2.84	87	1.52	219	17.5	9.8	10.2	8.5	125
C	28	63.5	8.1	3.22	3.16	102	1.52	257	20	9.3	9.1	9.5	150
D	45	71.7	7.1	2.98	3.18	95	1.36	224	21	8.5	9.8	8.1	113
E	18	60.0	7.9	2.92	2.90	85	1.32	197	20	8.4	9.2	7.1	118
Mean	..	..	7.6	3.12	3.00	94	1.41	226	19.1	9.3	10.2	8.8	125

Subjects A and B received ether-fractionated albumin. Subjects C, D, and E received electrophoretically separated albumin. The percentage of the intravascular albumin pool katabolized per day was calculated from plasma activity curves (x) as well as from urinary ^{131}I excretion (y). Pool Ratio = Ratio of extravascular to intravascular albumin.

TABLE II.—Comparison of Albumin Distribution and Turnover in Healthy African and European Males (Freeman *et al.*, personal communication of unpublished data)

			Intra-Vascular Albumin (g.)	Pool Ratio	Total Exchangeable Albumin (g.)	Half-life (Days)	Albumin katabolism		
							% per Day (x)	g. per day	mg. per kg. per Day
European (4)	..	{	Mean	1.21	290	21.5	7.6	10.1	147
			Range	1.08-1.35	274-302	19-23	7.3-8.2	8.6-11.5	121-163
African (3)	..	{	Mean	1.40	226	21	8.7	8.3	127
			Range	1.32-1.52	197-257	20-21	8.4-9.3	7.1-9.5	113-150

x = % of intravascular pool katabolized per day (calculated from plasma activity curves).

Compared with a group of normal Europeans the Africans have a consistently smaller intravascular pool, a consistently higher ratio of extravascular to intravascular albumin, and possibly a slightly lower rate of albumin katabolism.

Dr. A. S. McFarlane, of the National Institute for Medical Research, London, kindly supplied samples of iodinated albumin. The advice of Dr. McFarlane and of Dr. Christine Matthews is gratefully acknowledged. We are indebted to Professor Joseph Gillman for his interest in this work; to Professor J. F. Murray, South African Institute for Medical Research, who provided some laboratory facilities; and to the superintendent, Baragwanath Hospital, for permission to publish this report. Special thanks are due to the African volunteers whose sustained co-operation made these tests possible.

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FOETAL ASPHYXIA ASSOCIATED WITH UMBILICAL CORD AROUND THE NECK

BY

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Coiling of the umbilical cord around the foetal neck is a common complication of labour, said to occur about once in every five deliveries (Brews and Bender, 1955). Foetal asphyxia, deflexion attitudes, and malpresentations are sequelae of this complication recognized by most British and American textbooks of the present day, although generally this subject receives only scant attention. Shui and Eastman (1957), however, found no increased perinatal mortality attributable to this phenomenon in their recent series.

To find the cord coiled five times around the neck is relatively rare: this rarity and the severe foetal distress associated with it have prompted recording of the following case.

Case Report

A 35-year-old woman, married three years, attended University College Obstetric Hospital in her first pregnancy in 1956. She was a healthy woman with no past history of disease, found to be rhesus-positive and W.R.-negative. The expected date of delivery was calculated to be July 7, 1957. Pregnancy proceeded normally, uncomplicated by hyperten-

sion or pre-eclampsia. The foetus presented by the breech at the 28th week, but had undergone spontaneous version to a vertex presentation by the 32nd week. It remained thus and the foetal head was engaged at 36 weeks. The maternal pelvic cavity and outlet were adequate.

Labour began spontaneously at the 39th week with contractions. On her admission at 9 p.m. on June 30 the foetal head was in the upper pelvic cavity, and the foetal heart was regular at 132 beats a minute. The cervix was not taken up and was only 2 cm. dilated. Contractions were weak, but became strong and regular during the next two hours. Intramuscular pethidine, 125 mg., was given at 11 p.m. At 3.30 a.m. on July 1 the membranes ruptured spontaneously and copious amounts of thick green meconium escaped. The foetal heart remained steady and regular. Vaginal examination showed that the head was now deeply engaged, with the sagittal suture transverse; no cord was felt. The cervix was 8 cm. dilated. At 4 a.m. the foetal heart was first found irregular: there were coupling of the beats and variations in rhythm and rate, which was between 98 and 164 beats a minute. Uterine contractions were strong and regular, and the patient was given a further 125 mg. of pethidine. She was also given oxygen to breathe intermittently from a B.L.B. mask.

The cervix was fully dilated by 7.30 a.m.: the foetal heart now varied between 100 and 180 beats a minute. Preparations were made for forceps delivery, and the patient was premedicated with atropine, 1/100 gr. (0.65 mg.). General anaesthesia was induced with cyclopropane and maintained with gas, oxygen, and ether. Vaginal examination showed the foetal head at the level of the ischial spines in the left occipito-lateral position. After a right medio-lateral episiotomy, Kielland's forceps were applied by the wandering method and the foetal head was rotated and delivered. The foetal cord, devoid of pulsation, was coiled tightly five times around the neck and had to be severed before delivery of the trunk. Ergometrine, 0.5 mg., had been given intravenously with the crowning of the head, and the placenta and membranes were delivered intact by the Brandt-Andrews technique, with a total blood loss of 255 ml. The puerperium was uneventful.

The placenta weighed 740 g. and was normal. The cord was eccentrically implanted and its total length was 131 cm. (52½ in.).

The infant, a male, was limp and shocked at birth; although he gasped immediately, regular respirations were not established for 10 minutes, and the first cry was at 20 minutes. Resuscitation consisted in the clearing of the air passages, which contained thick green meconium, and the administration of gastric oxygen, and 0.25 mg. of nalorphine hydrobromide (*n*-allylnormorphine; "lethidrone") into the umbilical vein. The birth weight was 6 lb. 6 oz. (2,890 g.).

The baby was transferred to the sick-baby nursery and treated with intramuscular penicillin, 100,000 units, and streptomycin, 60 mg., twice daily for five days as well as one injection of "vikastab," 1 mg. Two hours after delivery he was found to be restless and irritable, with a shrill cry, marked opisthotonos, and exaggerated limb tone. He was sedated with intramuscular phenobarbitone, 1/8 gr. (8 mg.) four-hourly for two days. Respirations were shallow for the first 24 hours, but regular after that.

Feeding was started with expressed breast milk on the second day. By the fifth day the baby was established on the breast and transferred to the mother's ward. He was discharged with the mother, in good condition, fully breast-fed, on the 10th day, when the weight had risen to 6 lb. 14 oz. (3,120 g.). At the follow-up clinic six weeks later the baby was in satisfactory condition and weighed 7 lb. 14 oz. (3,570 g.), being totally breast-fed.

Arrangements were made for further follow-up visits, to ascertain whether any permanent cerebral damage might have resulted from the anoxia at birth.