Activation Analysis

Detailed information on the chemical composition of the body remains for the most part tantalizingly out of reach. Direct chemical analysis of normal adult cadavers is restrained by formidable difficulties and has seldom been achieved. Indirect methods, including balance studies and dilution techniques (with radioactive or stable tracers), give information, but we still have no reliable way of estimating, for example, the total amount of sodium or calcium in the body.

The method of activation analysis has many applications in biology and medicine.[1 2] In a novel use recently reported by G. W. K. Donaldson and colleagues at page 585 of the *B.M.J.* this week a familiar radioisotope test is replaced by a stable-tracer technique avoiding radiation to the patient.

Activation analysis offers unusual possibilities for the chemical analysis of the living person. As normally practised, this procedure requires exposure to bombardment by neutrons or other subatomic particles or radiations, followed by assay of the induced radioactivity, which is highly specific of the chemical elements contributing to it. In vitro it is a remarkably sensitive detector of many elements. Irradiated tissue will of course display many different radioactive substances, but simple chemical manipulation allows the desired isotope to be separated. Activation analysis in vivo is generally less sensitive, because the radiations of a single element are difficult to isolate from the confused spectrum of induced radioactivity. Other limitations are associated with the problem of securing uniform irradiation throughout the body and with the health hazard to the person irradiated.

Thermal (that is, low-energy) neutrons are the most effective for the induction of radioactivity but are unfortunately so heavily absorbed in body tissue that uniform irradiation cannot be attained. Fast neutrons penetrate adequately but are less effective in inducing radioactivity. However, if a stream of fast neutrons is directed on to the body some of them will, in passing through the tissues, be reduced to thermal energies and will therefore generate significant amounts of radioactivity.

The first application of this technique to living persons was reported in 1964 by J. Anderson and colleagues,[3] who used neutrons of energy 14 MeV from a small generator. This work has now been extended by Dr. M. J. Chamberlain and his colleagues, who report on it in two papers this week (pages 581 and 583). They used neutrons from a cyclotron in the physics department of Birmingham University to irradiate cadavers and living persons with the object of measuring total body sodium and calcium. Though a readily detectable signal can be obtained from calcium-49 after neutron irradiation, the accurate estimation of total body calcium is not possible. Its uneven concentration within the body and the skeletal variations among individuals present substantial difficulties in the analysis. Activation analysts can often use an internal standard to circumvent the problems created by non-uniform distribution and irradiation of an element in a sample. This technique has been used in the estimation of thyroid iodine,[4] but through lack of suitable isotopes is not applicable to calcium.

Chamberlain and colleagues consider that comparative measurements on individual patients at suitable intervals would help the understanding and management of certain bone disorders. H. E. Palmer and colleagues,[5] who have irradiated a number of cadavers with cyclotron-produced neutrons, suggest that a change of 2% in total body calcium could be detected by activation analysis. They consider also that an accuracy of 8% could be attained in a single determination of total body calcium, though they acknowledge that absolute calibration of the method would require the irradiation, ashing, and chemical analysis of several cadavers.

Surprising though it may appear, the sodium content of the body is still not known. The commonly used figure of 105 g. for a 70-kg. man, based on analysis of tissue samples from cadavers, is almost certainly too high. In the three living persons to whom Chamberlain and colleagues applied activation analysis the average sodium level was 49.6 mEq per kg. body weight, corresponding to a total content of about 80 g. The average value of exchangeable sodium, measured by an isotopic dilution technique in the same subjects, was equivalent to about 66 g. in a person weighing 70 kg. The difference of 18% between total body sodium and exchangeable sodium accords with the hypothesis, supported by some experimental studies, that an appreciable fraction of the sodium in the body is not rapidly exchangeable. About one-third of the total body sodium occurs in bone, and about half of this fraction is apparently isolated from the exchange processes which form the basis of isotopic dilution techniques.

The estimation of whole body sodium by activation analysis may be subject to error. Non-uniform distribution of sodium in the body brings (admittedly to a lesser degree) some of the uncertainties associated with the estimation of total body calcium. Further experiments are desirable also to assess the value of interesting suggestions made by Palmer and colleagues[5] for the estimation of nitrogen and phosphorus by activation analysis in vivo.

Whole body neutron irradiation of living subjects has so far been applied only to volunteers in the two British experimental teams and has involved modest (though not negligible) radiation doses. Ethical problems must be associated with neutron irradiation of patients, and they will need careful study if the full benefits of this technique are to be obtained.

Pigmented Gut

Pigmentation of the alimentary tract is a striking condition readily recognized by pathologists. It occurs in two conditions which are clinically and pathologically different, melanosis coli and lipofuscinosis.

Melanosis coli was first described over a hundred years ago. It is a brownish-black discoloration of the mucosa of the large bowel caused by deposition of melanotic pigment in the mononuclear cells of the lamina propria. It remained a pathological curiosity until 1933, when the sigmoidoscopic appearances were described, and its association with constipation and the continuous use of anthracene laxatives such as cascara was noted.[1] The pigment is formed from anthraquinone in these purgatives and is deposited with protein in the mucosa. When the drugs are stopped it disappears in a few months. Melanosis coli is rarely seen today, presumably because these laxatives are not consumed in sufficient quantity.

Lipofuscinosis of the gut, or brown bowel syndrome,[2] was first thought to be a form of melanosis coli, but forty years ago was recognized to be clinically and pathologically distinct. Lipofuscin is deposited in small-bowel muscle, which at

[1] *Brit. med. J.*, 1967, **1**, 584.
[2] *Brit. med. J.*, 1967, **3**, 509.
[3] Anderson, J., *et al.*, *Lancet*, 1964, **2**, 1201.
[4] Lenihan, J. M. A., Comar, D., Riviere, R., and Kellershohn, C. *J. nucl. Med.*, 1968, **9**, 110.
[5] Palmer, H. E., Nelp, W. B., Murano, R., and Rich, C., *Phys. in Med. Biol.*, 1968, **13**, 269.

laparotomy or post mortem is found to be pigmented. Clinically, whereas patients with melanosis coli are constipated, those with lipofuscinosis usually have diarrhoea due to steatorrhoea. Malabsorption in these patients may be due to coeliac syndrome, chronic pancreatitis, fibrocystic disease of the pancreas, Whipple's disease, or jejunal diverticulosis. Lipofuscinosis is also seen in patients with hypoproteinaemia, usually due to protein-losing gastroenteropathy, and has been recorded in hepatic cirrhosis.[3] B. Fox[4] has recently described the features in three patients in whom pathological material was obtained at laparotomy and post mortem. Two of them had hypoproteinaemia. The pigment was deposited in large amounts in the smooth muscle of the small intestine, and was also found in blood vessels and macrophages of this organ. Post-mortem material from one patient showed lipofuscin in smooth muscle of the oesophagus and stomach, in the lymph nodes, various brain cells, hepatocytes and Kupffer cells of the liver, heart muscle, and in the Leydig cells of the testes.

Lipofuscins are a group of pigments formed by oxidation of cellular lipoids and lipoproteins. They have variable but characteristic staining properties, which, according to A. G. E. Pearse,[5] depend on the degree of oxidation and the type of lipid in the cell. Thus, although the histological appearance of lipofuscins may vary, they do seem to be a group of intimately related substances. In the liver lipofuscins are often known as ceroids, and are found in haemochromatosis, when they may form the organic matrix on which iron is deposited in the organ.[6] Brown atrophy of the heart, which occurs in a variety of terminal diseases, is due to lipofuscin in the cardiac muscle.

The intriguing question about lipofuscinosis is its cause. It may be due to vitamin-E deficiency, for animals fed diets deficient in this vitamin develop lipofuscinosis, as do some patients with vitamin-E deficiency and steatorrhoea.[7] Vitamin E is an antioxidant,[8] and it has been suggested that, when it is lacking, lipofuscins are formed by oxidation of cellular lipids. Other mechanisms involving mitochondria may be at work.[9] There is also a close association between vitamin E and protein metabolism, which may be a clue to the mechanism underlying the lipofuscinosis that is seen in patients with hypoproteinaemia.[10] As there is increasing evidence that vitamin-E deficiency occurs in various gastrointestinal disorders,[11 12] its role in producing deposition of lipofuscin in man needs to be fully investigated.

Lipofuscinosis does not have any specific clinical symptoms or physical signs, and there is no known treatment. Its cause and significance remain a challenge to the research worker, whether clinician, pathologist, or biochemist. Perhaps his efforts will be rewarded by a clear-cut answer, as was the case with melanosis coli and the anthracene purgatives almost forty years ago.

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Virus from Monkeys

Though fragmentary accounts of the disease acquired by laboratory workers in Germany and Yugoslavia from vervet monkeys have appeared, and the earlier laboratory findings been reviewed,[1] only now is a full clinical and epidemiological description of the disease available. G. A. Martini and colleagues[2] describe 23 cases which occurred in Marburg and W. Stille and colleagues[3] 6 cases in Frankfurt.

Estimates of the incubation period lie between four and nine days. There was a sudden onset of nausea, severe headache (mainly frontal or temporal), and tenderness of the eyeballs. During the first few days there was increasing fever, relative bradycardia, and pain and a feeling of tension in the trunk muscles and around the hips; vomiting was common. After one or two days there was often watery diarrhoea, with up to ten motions a day. As the fever subsided, from about the seventh day of illness, the vomiting decreased but the diarrhoea continued for several more days. In some patients there was a second febrile phase from the twelfth to the fourteenth days associated with myocarditis and orchitis. The severity of the diarrhoea in general paralleled the severity of the disease, though a few patients showed constipation, at least for a time. Dryness of the mouth was a common complaint, even before onset of diarrhoea and vomiting.

Between the fifth and seventh day of illness a rash appeared, most marked on the buttocks, trunk, and the outer aspects of the upper arms. At first there were red pinhead spots round the hair follicles; after about 24 hours the rash became maculopapular, then confluent. In severe cases there was a diffuse livid erythema on the face, trunk, and limbs, sometimes associated with cyanosis of the lips. Some cases had dermatitis of the scrotum or labia. In a few there were vesicles (on lips, abdomen, or thumb), some or all of which may have been due to recrudescent herpes simplex. After about the sixteenth day there was fine desquamation of the skin affected by the rash, especially on the palms, soles, forearms, and legs, lasting about two weeks. About half the patients had conjunctivitis at one time or another. There was also an enanthem which appeared a day before the rash in some patients—deep red discoloration and "sago-like" vesicles on the soft palate sometimes spreading to the hard palate. Some had inflamed tonsils with yellowish pinhead areas of exudate. Lymph nodes were small and not tender, but palpable, sometimes before the onset of the rash, in the neck and axilla. Two patients had signs of meningeal irritation, but the cerebrospinal fluid was essentially normal. Most patients were peevish and uncooperative at the height of the illness, but some had confusion, depression, or anxiety, and

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