alteration, which is mediated by a foreign environment, before they can be recognized. Being the antigens of fixed tissues, they only express themselves when, as in traumatic injury, they become displaced from their normal organ. Complete tolerance to modified tissue-specific antigens, such as the prevention of auto-immune disease by pre-treatment, has never been found to induce malignancy, nor in clinical cases do blood chimeras develop leukæmias. This may be because such cells possess recognition markers, although the host cannot detect them. Other [illegible] by the conglutination of dispersed sponges, and by the association together of like cells to like, when grown in tissue culture.

If the defence mechanism for the disposal of displaced normal autologous cells is in any way related to classical immune processes, then acquired tolerance to tissue-specific antigens cannot occur naturally. But it would appear to have been induced experimentally, for auto-immune disease can be prevented by neo-natal injection of the appropriate tissue. Paterson[13] used a spinal cord suspension to prevent the allergic encephalitis produced by nervous tissue plus adjuvant, and Chutna[14] used testes extract to prevent auto-immune aspermatogenesis. Using auto-immune techniques it has also been shown that embryonic and newborn rat nervous tissue is not paralytic for the adult, as these tissues do not develop specific antigens until a few days after birth[15]. Their time of appearance varies according to species, and follows the course of myelin development.

Cancer cells, however, at a certain stage of progression, do survive when displaced. Moreover, when acquired tolerance to their species and iso-antigens is induced, they survive just as well in the tissues of an alien strain or species. Such cells must lack surface factors which normally identify them, and a mass of evidence[4,5], direct or indirect, has revealed that they are in fact deficient in the tissue-specific antigens located in the microsome fraction, that is, in the surface membranes.

An interesting aspect of this work is that a mouse tumour growing in a rat could apparently—at least for a time—acquire the species specificity of its host, and fail, in the absence of tolerance, to grow in its host of origin. There is growing evidence[16–18] that the tumour cell surface incorporates environmental constituents which would, as in this case, presumably neutralize iso-antigens. We have previously suggested that acquired tolera[illegible] might be due to the induction of a univalent block antibody which continued to be produced on challe[illegible] when immunological maturity was reached. Now [illegible] know that the challenged fœtus can produce[20,21] antibod[illegible] yet at the same time acquire antigenic tolerance, [illegible] also that *in vitro* exposure to iso-antibodies produ[illegible] enhancement (that is, adult tolerance) of tumour growt[illegible] this suggestion becomes more plausible.

In the present experiments an inherited species to [illegible] tumour would no longer grow in its original [illegible] except under conditions of acquired tolerance, presuma[illegible] because rat protein had been incorporated in the [illegible] surface. These results are in line with the work [illegible] Molomut[22], who described a reversible homotranspla[illegible] ability, while we here describe a reversible heterotra[illegible] plantability.

The implications of these results, both to the mechan[illegible] of acquired tolerance and the now undoubted importa[illegible] of cell surface change as a crucial factor in the emerge[illegible] of the cancer cell, seem interesting.

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A UNIVERSALLY APPLICABLE METHOD FOR ASSAYING THYROID FUNCTION IN VERTEBRATES

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COMPARATIVE physiology needs a universally applicable method for measuring thyroid activity which is non-destructive and independent of size of animal. Thyroid function is at present measured in small vertebrates by histological appraisal of the height of the thyroid acinar epithelium[1]. More recently, the serum iodine-131 conversion ratio has been used for this purpose[2]. Both methods, however, usually involve killing the animal, because thyroid tissue or relatively large amounts of blood serum must be obtained. Although thyroid uptake of iodine-131 has been examined in mice and rats by small collimated sodium iodine crystal scintillometers[3], as commonly done clinically in man, this method is not applicable to more minute mammals or non-mammalian vertebrates, particularly aquatic species. A method of measuring thyroid function by determining thyroid iodine binding by whole-body assay[4] of retention of iodine-131 is reported here because it is non-destructive, requires a minimum of handling and restraint, and does not require collection of serum or excreta. It is a [illegible] independent of body size and shape and localization [illegible] thyroid tissue.

The method requires a scintillometer with a well la[illegible] enough to hold the entire body. For minute species [illegible] sodium iodide crystal well counter ordinarily used [illegible] assay serum samples can be used. For animals grea[illegible] than 5 cm. in length, either a tank-type liquid scintil[illegible] meter or two large, flat-surfaced sodium iodide cryst[illegible] are needed. In order to reduce to a minimum the imp[illegible] tance of the geometric localization of iodine-131, [illegible] animal is placed between two crystals in permanen[illegible] fixed position, or within the well of the tank-type liq[illegible] scintillometer. In the method described here, three liq[illegible] scintillometers, known respectively as the Los Alam[illegible] Small Animal Counter[5,6], *Humco I*[7] and *Humco I*[illegible] were used. The counter for small animals[5,6] consisted [illegible] a 35·5-litre stainless steel tank of 1,4-*bis*-2-(5-phenyl[illegible] azolyl)-benzene solution scanned by six 2-in. pho[illegible]

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multiplier tubes and the usual electronic scaling and pulse height analyser units. It had a horizontal well, 4·25 in. in diameter and 12 in. in depth, into which a quart tubular paper ice-cream carton containing the animal could be placed for assay. The tank was shielded by 1·5 in. of liquid mercury or 7 in. of armour plate in order to achieve a low background. Commercial models of this counter have recently become available. The counters used for large animals and man[7,8] are similar in principle and have been described recently in full detail elsewhere.

[illegible] as sodium iodide in water. This dose should be varied in relation to the individual counting apparatus so that a maximum counting rate can be achieved immediately after administration of the dose without coincident or mechanical loss of counts. The route of administration is chosen which suits best the species being studied. Intravenous dosage is unnecessary because gastric and peritoneal absorption of iodide is complete. Thus, the iodine-131 is given commonly by stomach tube or intraperitoneal injection to most animals. It can be administered in the drinking water or in the tank in which the fish or amphibians reside. In frogs, injection into the dorsal lymph sac seems the best route. The animals are assayed in the whole-body counter immediately after the dose is given or absorbed, and the total (or 100 per cent) dose is determined in whole-body counts per second for each animal. Individual whole-body retention curves are then determined by recounting the animals at successive intervals in the same manner. The necessary frequency of assay is determined by the rapidity of urinary excretion of unbound iodide in the species being studied. Since in most animals, including man, urinary excretion of unbound iodide has a half-time of 6–9 h. unbound iodine-131 sufficient in amount to raise the whole-body radioactivity

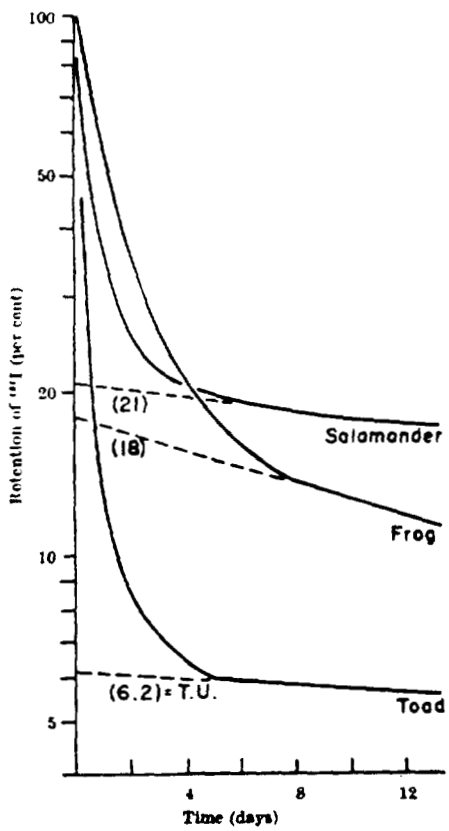

Fig. 1. Whole-body retention curves of iodine-131 in *A. tigrinum*, *R. catesbeiana* and *B. americanus*. The dotted lines extend the flat terminal portion of the curve to the ordinate, where the point of interception is considered the thyroid uptake percentage

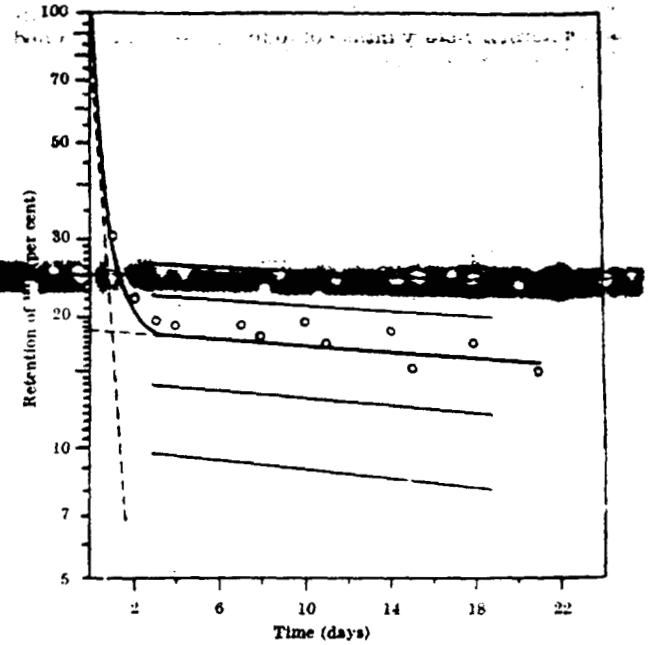

Fig. 2. Whole-body retention curve (heavy line) of iodine-131 in 26 normal human subjects and the two exponential regression lines (dotted) composing it. The thin lines parallel to the second component line (protein-bound iodine compartment) represent the 68 and 95 per cent fiducial limits of the fitted (heavy) line. Retention of iodine-131 within this area (10–27 per cent) is normal. The half-time regression rate in this compartment in man is 91·9 days

significantly is present for only 3–4 days[9,10]. After this time, whole-body radioactivity is due to bound iodine-131 in the thyroid and in the circulation[10]. Assays performed at daily intervals for one week are, therefore, frequent enough to determine the shape of the whole-body retention curve for most species. After this curve has been determined once for a species of animal, thyroid iodine uptake can then be determined by this method with only an initial (or 100 per cent) assay and a second one 3–4 days later. This economy of measurements is possible because the size and regression rates of the metabolic compartments which determine the shape of these curves for whole-body retention of iodide are typical for each species.

In Fig. 1, three whole-body retention curves for 1 μc. of intraperitoneally injected sodium iodine-131 are shown to illustrate typical results obtained by this method. These curves are fitted to the arithmetic means for the data obtained from whole-body counts of three tiger salamanders (*A. tigrinum*), four frogs (*R. catesbeiana*) and six toads (*B. americanus*). The terminal straight portion of each curve has been extrapolated back to zero time by the dotted line. The point of its intercept with the ordinate is considered the percentage of the whole dose that was bound to protein by the thyroid gland and is clinically called 'thyroid uptake'. The level of interception of this line can be lowered by feeding sufficient inert iodine to block thyroid function and, in some species, raised by pre-injection with thyroid-stimulating hormone. For the species examined here, the mean thyroid uptake was: salamander, 21 per cent; frog, 18 per cent; and toad, 6 per cent. By this method, normal man has a thyroid uptake within the range of 10–27 per cent, with a mean of 18·47 ± 0·93 per cent[4] (Fig. 2).

Hypothyroid man has a thyroid activity remarkably similar to that of the toads measured in this examinations. The slopes of the terminal straight portion of the retention curve indicate that the rates of utilization of the protein-bound or hormonal iodine differ widely in these species. The slope for the toad approximates that for normal man, where the regression formula for this exponential portion of the curve has been found to indicate a turnover rate

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of hormonal iodine of 7·7 per cent a day[4]. Homeostasis and a normal dietary intake of iodine must be maintained during such observations if the true slope for hormonal utilization rate is to be determined accurately by this method.

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HEAT-LABILE COMPLEMENT-FIXING ANTIGENS IN THE GROWTH-CYCLE OF FOOT-AND-MOUTH DISEASE VIRUS

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IT has been shown by differential centrifugation[1] and agar diffusion[2] that foot-and-mouth disease virus suspensions contain two components which fix complement with type-specific antiserum. The two components behave differently on heating at 56°. The 25-mμ infective component is broken down to its ribonucleic acid and a component serologically indistinguishable from the naturally occurring small component[3,4] with an approximate doubling of the complement-fixing activity, whereas the smaller, non-infective, component appears to undergo relatively minor changes. Bradish and Brooksby[4] showed, however, that the small component, prepared from guinea pig pad epithelium by ultra-centrifugation at 45,000 r.p.m. for 54 min, lost about 25 per cent of its complement-fixing activity on heating at 56°. They considered this lability to be a normal property of the small component. In the experiments described here, we have found that the separated 25-mμ component as well as the small component possesses some complement-fixing activity which is destroyed by heating at 56°, the proportion of labile and stable activity depending on the time at which the virus is collected after infection.

Heat-labile complement-fixing activity has been observed in all the different sources of virus we have so far examined. With unfractionated virus suspensions prepared from the leg muscles of 7 days old mice paralysed after intraperitoneal inoculation of the virus and the harvests prepared from suspended cultures of bovine tongue epithelium fragments infected with the virus, the proportion of heat-labile complement-fixing activity was much greater than we have normally encountered with guinea pig pad vesicle fluid. In some of the tongue epithelium cultures collected 18 h after infection, as much as 80 per cent of the complement-fixing activity was destroyed by heating at 56° for 1 h.

Efforts have been made to separate the heat-labile component from the 25 mμ and small components of the

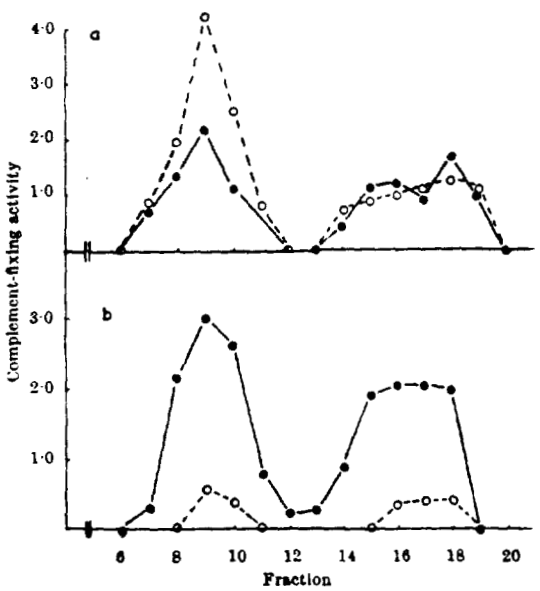

Fig. 1. Separation of foot-and-mouth virus suspensions by [illegible] density centrifugation. The suspensions were layered over 15-[illegible]cent sucrose in 0·01 M phosphate, pH 7·6, and centrifuged at [illegible] r.p.m. for 120 min in the *SW*-39 head of the Spinco ultra-cent[illegible] The figure shows the separation of (*a*) guinea pig vesicular flu[illegible] (*b*) bovine tongue epithelium cultures. Each fraction contained [illegible] The ordinate shows ml. 1/30 complement fixed by each fr[illegible] ●, Unheated; ○, heated at 56°

epithelium culture showed a loss of complemen[illegible] activity on heating at 56° (Fig. 1*b*). These results su[illegible] that the properties of the two components mi[illegible] influenced by the time at which they were c[illegible] from the infected tissues.