ments of the earth from naturally occurring deposits of high concentration to states of low concentration dissemination. Yet despite this, it will still be physically possible to stabilize the human population at some reasonable figure, and by means of the energy from sunshine alone to utilize low-grade concentrations of materials and still maintain a high-energy industrial civilization indefinitely.

Whether this possibility shall be realized, or whether we shall continue as at present until a succession of crises develop—overpopulation, exhaustion of resources and eventual decline—depends largely upon whether a serious cultural lag can be overcome. In view of the rapidity with which the transition to our present state has occurred it is not surprising that such a cultural lag should exist, and that we should continue to react to the fundamentally simple physical, chemical, and biological needs of our social complex with the sacred-cow behavior patterns of our agrarian and prescientific past. However, it is upon our ability to eliminate this lag and to evolve a culture more nearly in conformity with the limitations imposed upon us by the basic properties of matter and energy that the future of our civilization largely depends.

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Analysis of Microcomposition of Biological Tissue by Means of Induced Radioactivity

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RADIOACTIVE ISOTOPES HAVE FREQUENTLY been used for the determination of reaction rates and distribution of chemical elements and compounds in the animal body; for example, the measurement of the total body water or the sodium space (*6, 7, 9*). Some of the radionuclides, however, cannot be used for determining the concentrations of their stable isotopes in tissue because the distribution of the administered radioactive tracer sample will not equilibrate, owing to absorption and excretion. On the other hand, the radioactive isotopes can be used to indicate the rate of turnover of substances in biochemical reactions. There are many important problems concerning "trace elements"[2] (e.g. see *11*) which involve knowledge of their distribution. The microchemical and spectroscopic methods of analysis which are now in use are not quantitative for most elements below 10^{-6} g/sample. It would be desirable to have methods of analysis which are accurate to well below this quantity. It seems certain that by using more highly sensitive methods of analysis much new information could be obtained concerning the biochemistry of trace elements in normal or diseased animals and plants.

Seaborg and Livingood (*10*) in 1938 solved a somewhat similar problem in their attempt to analyze for traces of gallium in iron. They exposed the gallium-contaminated sample to deuterons and determined the amount of gallium impurity by measuring the induced radioactivity. The use of this technique for biological studies was reported by us in 1947 (*12*) and later the same year by Brues and Robertson (*2*), who had used the technique independently. Clark and Overman (*3*) named the procedure "activation analysis." It may be used for biological research in more than one way. To learn the amount or concentration of

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[2] "Trace" elements are stable chemical elements, the presence of which, in small quantities, is essential for the lives of plants and animals. "Tracers" are radioactive or stable isotopes of elements which are suitable for study of the biochemical and physiological role of these elements or their compounds.

various trace elements, one may take a sample of the tissue or tissue extract and, after proper processing, expose this sample to a source of nuclear particles, e.g. in a pile or cyclotron. Several of the elements present in this sample may become radioactive, and, after a known exposure, the amount of each newly formed radioactive isotope may be measured by standard radiochemical techniques. If a radioactive isotope A is formed from trace element B, the mass of the element B originally present in the sample may be calculated from the formula:

$$x = \frac{d \cdot A \cdot \varepsilon^{\lambda \cdot \tau}}{6.02 \cdot 10^{23} \cdot \sigma \cdot F(1 - \varepsilon^{-\lambda \cdot t})} \quad (1)$$

where: x = unknown mass; A = atomic weight; F = particle flux; λ = decay constant of induced radioactivity; σ = cross section of B to form A; t = length of time of exposure to neutrons; τ = length of time elapsed between neutron exposure and time of measurement of rate of disintegration of the chemically separated sample A; and d = rate of disintegration of sample A measured at time τ.

The most convenient radiations to use are thermal neutrons produced by one of the chain-reacting piles. The above formula will hold true if the neutron flux remains constant throughout irradiation time, if the total amount of substance in the sample is not large enough to depress appreciably the neutron flux, and if the irradiation is not intense enough to significantly decrease the amount of isotope B to form isotope A. One does not always have to determine the absolute neutron flux in such a case. It is more convenient to measure the radioactivity induced after simultaneous irradiation of a known mass of element B. If neutrons are used as the activating agent, there are some 50 elements of biological importance which have large enough cross sections to make this technique an extremely sensitive one. Some of these elements are listed in Table 1.

Activation analysis may be also used for the study of distribution and metabolism of elements introduced into an organism in a manner somewhat analogous to radioactive tracer technique. Thus, for example, an element may be introduced into the circulation of an animal, and its distribution, excretion, etc. may be observed at some later time. Experiments of this kind may not be legitimately called tracer experiments because the amount of the element added to

TABLE 1

ESTIMATED DETECTION LIMIT OF ACTIVATION ANALYSIS FOR A FEW ELEMENTS, USING THERMAL NEUTRON CAPTURE REACTION*

Symbol of element	Atomic No. (Z)	Atomic Wt (A)	Natural atom cross section ($\sigma \times 10^{24}$ in cm^2)	Half life of induced activity (T)	Length of exposure (t)	Time elapsed after exposure (τ)	Amt giving 10 sec^{-1} disintegrations (gm)
Na	11	23	0.63	14.8 hr	5 T	T	2.4×10^{-9}
P	15	31	0.23	14.3 d	5 T	½ T	6.4×10^{-9}
K	19	41	0.067	12.4 hr	1 T	5 T	4×10^{-8}
Ca	20	44	0.013	180 d	0.02 T	T	2.2×10^{-7}
Sc	21	45	22.0	85 d	0.2 T	2 T	10^{-10}
Cr	24	50	0.5	26.5 d	0.4 T	3 T	5×10^{-9}
Mn	25	55	10.7	2.59 hr	1 T	5 T	1.8×10^{-9}
Fe	26	58	0.001	47 d	0.2 T	5 T	2.5×10^{-6}
Cu	29	63	2.0	12.8 hr	1 T	5 T	2.1×10^{-9}
Zn	30	64	0.26	250 d	⅕ T	⅖ T	4×10^{-8}
As	33	75	4.2	26.8 hr	1 T	5 T	1.2×10^{-9}
Br	35	81	1.11	34 hr	1 T	5 T	4.8×10^{-9}
Mo	42	98	0.1	67 hr	1 T	5 T	6.5×10^{-8}
Rh	45	103	137	44 sec	1 T	5 T	0.4×10^{-10}
Ag	47	109	1.1	225 d	0.05 T	0.3 T	1.6×10^{-8}
In	49	113	2.52	48 d	0.5 T	2 T	2.8×10^{-9}
Sb	51	121	3.8	2.8 d	1 T	5 T	2.4×10^{-9}
Te	52	126	0.15	9.3 hr	1 T	5 T	5.5×10^{-9}
I	53	127	6.25	25 min	1 T	5 T	1.3×10^{-9}
Cs	55	133	25.6	1.7 yr	0.002 T	0.1 T	2×10^{-9}
Ba	56	138	0.367	86 min	1 T	5 T	2.5×10^{-9}
Dy	66	164	725	140 min	1 T	5 T	0.15×10^{-10}
Pt	78	196	1.20	3.3 d	2 T	5 T	2.2×10^{-8}
Au	79	197	96.4	2.7 d	2 T	5 T	2.7×10^{-9}
Hg	80	202	0.725	51.5 d	0.2 T	T	2.6×10^{-8}

* Assumed neutron flux, that of the Clinton pile (8), $F = 5 \times 10^{11}$ cm^{-2} sec^{-1}; assumed disintegration rate necessary for identification and measurement of each radioisotope, $C = 10$ sec^{-1}.

the organism must always be larger or of the same order of magnitude as the amount already present. The metabolism of the element, therefore, may be altered during the process. Nevertheless, valuable data may be obtained regarding distribution and metabolism of minute quantities of certain elements. In some instances, the use of radioactive isotopes for such purposes requires that the radiation dose delivered to the tissues during the experiment be small to avoid disturbing biological functions. If certain tissues concentrate or selectively absorb the isotope, it is sometimes hard to avoid high dosage of such tissues. To be able to measure the concentration of the isotope in other parts of the body, one must administer a large dose. In addition, some of the radioactive isotopes these is the flux of particles. Using the published value for the flux density of the Clinton pile (*8*), the sensitivity of the method can be calculated for some elements to be as much as 1,000 times greater than that by any other known method. Other advantages are that one can determine the amounts of several elements present in the same sample simultaneously and, finally, that the method has a high specificity for certain elements. One should emphasize that the method is quantitative and that it will not require special techniques for individual elements except those of radio-chemistry.

In order to test the practicability of the ideas outlined above, some experiments were done using elements whose chemistry is easy and well known. Since

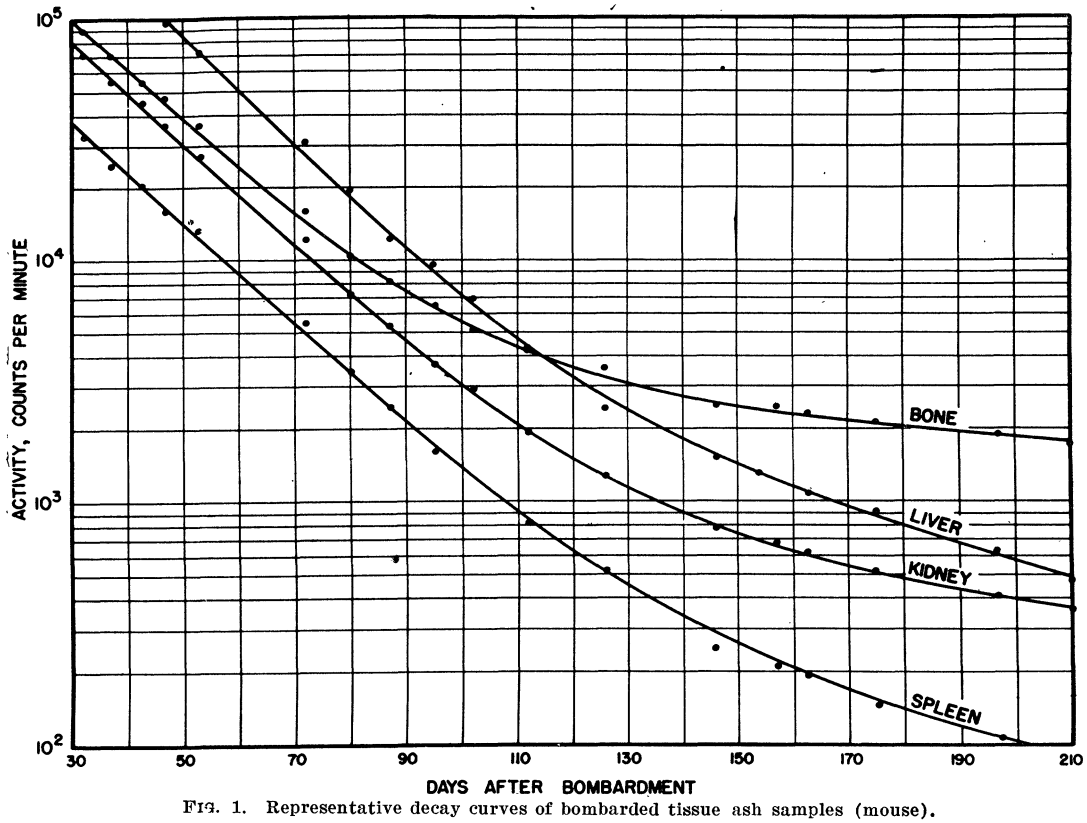

FIG. 1. Representative decay curves of bombarded tissue ash samples (mouse).

have too short half lives to permit their prolonged observation in animals; one would have to give a large dose of such a tracer in order to allow for its decay during the experiment. On the other hand, activation analysis does not have these limitations in measuring distribution of elements.

One of the most attractive features of the method described in this paper is its extreme sensitivity. Formula 1 indicates the factors influencing this sensitivity, and one may say that the most important of gold has an unusually large cross section for neutrons and since it is being used in this laboratory in connection with the study of rheumatoid arthritis (*1*), it appeared to be worth while to start with this element. The technique of its separation, deposition, and counting has been described by one of us (*5*). One normal female mouse, age six months, was injected in the tail vein with 10 μg of stable Au in the form of the soluble sodium gold thiosulfate salt. Upon autopsy and dissection a group of 19 repre-

sentative tissue samples was selected. Each sample was wet ashed, then irradiated by slow neutrons in a pile.[3] After irradiation and a suitable cooling period, the ash was dissolved in aqua regia. A small portion was used to prepare samples for counting. Typical decay curves from the counting of the latter are to be found in Fig. 1. Since counting began about one week after irradiation, some of the important short-lived isotopes are not present in these curves (Na^{24}, K^{42}, etc.) The effective half life of several of the tissue ash samples was found to be between 14 and 15 days, which would indicate that the principal activity is due to P^{32}. By using the value of the counting rates obtained on the fourth day after removal from the pile, the relative activities of several of the samples were determined. These data, for the tissues of the mouse, are shown in Table 2, where the samples are listed according to decreasing radioactivity per mg of wet tissue. Also listed are the activities per mg of estimated dry ash. The radioactivity per unit wet weight is roughly proportional to the phosphorus content of each organ, while the relative activity per mg dry ash weight is almost constant for most organs, indicating that the ash has an approximately constant percentage of phosphorus. At the end of 90 days the radioactivity of phosphorus decreased sufficiently to make some of the longer-lived components appear.

TABLE 2

RELATIVE RADIOACTIVITY OF IRRADIATED ASH ONE WEEK FOLLOWING EXPOSURE TO THERMAL NEUTRONS

Tissue	Wet wt (mg)	Estimated ash wt (mg)	Relative activity*/ mg of ash wt
Bone	29.8	7.9	1,000
Pancreas	179.6	3.9	1,500
Kidneys	305.6	3.9	1,720
Thymus	61.8	0.8	1,660
Spleen	141.4	2.7	1,110
Liver	1,458.8	57.0	500
Brain	436.1	6.8	1,220
Heart	107.2	1.1	1,780
Lung	175.2	2.1	1,500
Ovaries	21.8	0.3	1,110
Adrenals	11.6	0.2	830
Lymph nodes	30.8	0.2	1,660
Muscle	70.4	0.9	1,060
Red cells	0.15 (ml)	3.0	195
Gall bladder	14.6	0.1	830
Skin	101.6	5.1	145
Tendon	5.4	0.4	78
Gut	3,832.7	64.0	244
Plasma	0.15 (ml)	3.0	11.1
Control sample tube (empty)	very small	very small	.....

* Mean of measurements on two samples.

[3] Curtis and Teresi have previously demonstrated various radioactivities induced in tissues by neutrons (4).

Another portion of the solution was used to make radiochemical separations of various elements, in order to study the distribution of these elements and the administered gold. The first part of this work, namely, the study of the distribution of gold, is now complete. The activated gold was separated from each sample by precipitating it, with carrier, as the metal. The activities were determined after electroplating on platinum planchets. The half life of each sample was calculated. The values obtained showed

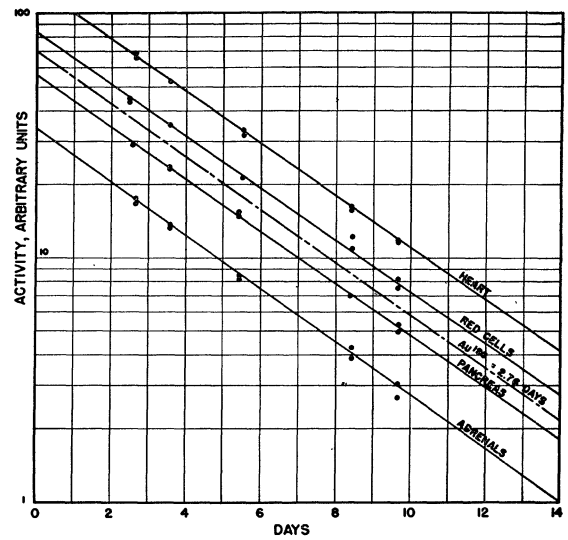

FIG. 2. Representative decay curves of Au^{198} recovered from tissue samples which were wet ashed and then bombarded by neutrons.

that excellent separation of the Au^{198} activity had been obtained. Typical decay curves are shown in Fig. 2. Table 3 gives the results of the distribution of radioactive gold. To some extent, these parallel the data obtained with mice injected with Au^{198}. Twenty-three per cent of the gold injected was recovered, which indicates that the rest of it was excreted in the one-month period. In the determination of the gold distribution a monitoring sample was used. This was prepared by simultaneously irradiating 10 μg of pure gold, as the chloride, with the same flux of neutrons as was received by the samples of mouse tissue. The activity of this gold sample was also used to calculate the neutron flux of the pile, since the cross section of Au^{197} for neutron capture is known.

In another experiment 20 ml of blood from a leukemic patient was fractionated into plasma, white cells, and red cells. These three ashed samples were also irradiated in the pile. The activity of the blood fractions, shown in Table 4, indicates that the relative radioactivity induced in the white cells per mg of dry

ash weight is about 10 times as high as that induced in red cells, or 50 times as high as the radioactivity induced in the plasma. Our attention is now focused on isolating the several isotopes which are responsible for the white cell radioactivity. The powerful possibilities which this technique offers are clearly illustrated in Table 5, which lists the Au found in the blood of a leukemic patient. The computations give 14 μg of gold for the total white cell volume of the body. To determine this small amount, one need use only about 10 cc of blood.

TABLE 3

DISTRIBUTION OF GOLD IN A MOUSE*

	Mass of organ (gm)	Amt of Au^{197}/gm of wet tissue (gm)	Total amt of Au^{197}/organ (gm)
Monitor gold sample	.....	.....	10×10^{-6}
Liver	1.458	4.4×10^{-7}	640×10^{-9}
Ovary	0.022	4.3×10^{-7}	9.5×19^{-9}
Thymus	0.062	3.2×10^{-7}	19×10^{-9}
Adrenals	0.012	3.1×10^{-7}	3.6×10^{-9}
Lung	0.175	2.6×10^{-7}	46×10^{-9}
Lymph nodes	0.031	2.2×10^{-7}	6.8×10^{-9}
Spleen	0.141	1.7×10^{-7}	24×10^{-9}
Kidney	0.306	1.6×10^{-7}	49×10^{-9}
Heart	0.107	1.5×10^{-7}	16×10^{-9}
Bone	2.600	1.1×10^{-7}	290×10^{-9}
Pancreas	0.015	1.1×10^{-7}	1.6×10^{-9}
Skin	3.100	1.0×10^{-7}	310×10^{-9}
Gall bladder	0.015	1.0×10^{-7}	1.5×10^{-9}
Tendon	0.050	0.7×10^{-7}	3.5×10^{-9}
Muscle	11.20	0.65×10^{-7}	730×10^{-9}
Red & white cells	1.05	0.65×10^{-7}	69×10^{-9}
Brain	0.502	0.2×10^{-7}	10×10^{-9}
Gut	3.6	0.09×10^{-7}	32×10^{-9}
Plasma	1.0	0.05×10^{-7}	5×10^{-9}
Total	25.446	.....	2276.5×10^{-9} gm or ⌣ 23% of total injected

* 10 μg of stable gold administered intravenously to a mouse in the form of gold sodium thiosulfate.

Mouse sacrificed 30 days after administration, tissues wet ashed and irradiated in the Hanford pile.

Subsequently the gold was removed by radiochemical methods, and its half life and beta-ray energy verified and counted in duplicate samples.

TABLE 4

RELATIVE RADIOACTIVITY OF IRRADIATED BLOOD ASH FROM A PATIENT WITH LYMPHOID LEUKEMIA (One week after exposure to neutrons)

Tissue	Estimated ash wt used in determination (mg)	Relative activity* / mg of ash wt
Red cells	114	1
White cells†	8	11
Plasma	280	0.2

* Mean of measurements on two samples.

† The white cells were prepared by centrifuging with beef albumin.

These considerations and experimental data indicate that microanalysis of tissue constituents by induced radioactivity may become a useful technique for the determination of ultramicro amounts of a number of elements. It is expected that the technique will play an important role not only in tracer biochemistry but in plant nutrition, pharmacology, and toxicology as well.

TABLE 5

DISTRIBUTION OF GOLD IN HUMAN BLOOD FROM A LEUKEMIC PATIENT

	Estimated wet mass in body (gm)	Gm of Au^{197}/gm of wet mass	Total Au^{197} in circulation of person (gm)
White cells	130	11×10^{-8}	14.3×10^{-6}
Red cells	2,600	0.4×10^{-8}	10.5×10^{-6}
Plasma	2,650	0.07×10^{-8}	18.5×10^{-6}

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