the source of material. It was found that the added phenol could be completely extracted with ether without interfering with the subsequent insulin reaction. Deviation between concentration and turbidity before iodination was especially observed with the partially inactivated insulin. The characteristics of the iodination curves obtained in this manner were relatively constant and reproducible.

The iodination of insulin or other proteins also proceeds rapidly in the absence of starch. However, uniformity of results and the stability of the suspension of iodinated proteins were found to be improved in the presence of starch prepared as described.

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TRACER STUDIES WITH RADIOACTIVE SODIUM IN PATIENTS WITH PERIPHERAL VASCULAR DISEASE[1, 2]

IN patients suffering from peripheral vascular diseases, the circulation time, volume of blood circulating in the extremities, and relation between plasma and extravascular fluid in the extremities may be altered. A study of these should be of diagnostic and prognostic value. The employment of radioactive sodium as a tracer substance in the blood is well suited to this purpose. It is easily prepared in the cyclotron, has a rather short half life (14.8 hours) and emits radiations which are readily detected. The injection of a few cu cm of normal saline into the blood stream can have no physiological effect, if the accompanying dose of radiation is insignificant. In the experiments here reported, this was the case.

The radioactive isotope is prepared by bombarding sodium metaborate in the cyclotron with deuterons. The metaborate containing the active atoms is dissolved in water, acidified with hydrochloric acid, and treated with methyl alcohol. The resulting methyl borate and water are driven off by heating, leaving sodium chloride. This is prepared in sterile solution in the desired concentration for injection.

When this material is injected into a vein it is carried throughout the vascular system, and the time required for it to reach a foot can be determined by placing a Geiger-Müller counting tube at the extremity and timing the interval between injection and audible registration by the counter. Since there is constant interchange of sodium between plasma and extravascular fluid, the amount of radio-sodium in the foot will increase until equilibrium is established between these two. The manner in which this equilibrium is built up depends on the volume of blood traversing the region per unit time, and on the relation between intra- and extra-vascular space; these are related to the normal or diseased condition of the foot. By following the counting rate during a period after the administration of the isotope, information can be gained regarding this build-up.

The experimental procedure is as follows: The syringe containing 60–200 micro-curies of radiosodium in 3–10 cu cm of saline is held close to the site of the injection, the Geiger-Müller tube, in a lead housing with a thin aluminum window, is placed against the sole of the patient's foot, and the scaling device on the counter adjusted so that 1 or 2 clicks are heard each 5 seconds. The injection and the electric stop-clock are started simultaneously, and counts recorded every 5 seconds, the time of the end of the injection also being noted. When the material reaches the foot, the number of clicks per 5 seconds increases definitely and sharply; the 5-second counting is, however, carried on for about a minute longer, to be sure that no erratic background has been mistaken for the arrival of the material. After this, the counting is carried on minute by minute as the build-up proceeds. After 10 or 15 minutes the counter is shifted to the other foot. Subsequently measurements are made at various regions of the legs, hands, etc., with frequent return to the feet.

Circulation times, arm to foot, have been determined in 35 individuals; the average value was 45 seconds, but the range was considerable. The highest was 90 seconds, in an elderly diabetic, arteriosclerotic woman who was fibrillating at the time of the test. The lowest was 15 seconds, in a young man in a highly nervous state, with a pulse of 100, suffering from scleroderma. In ten individuals considered to be normal as far as circulation was concerned, eight fell between 45 and 55 seconds; the other two were 60 seconds. Five cases of thromboangitis obliterans were all below 35 seconds. Seven arteriosclerotics showed a range between 30 and 80 seconds; it may be possible later to correlate circulation time with stage of disease. Four cases with ulceration and inflammation (three diabetics) showed times below 35 seconds.[3]

Probably more promising than circulation time as a clinical aid is the curve of equilibrium build-up. Fig. 1 shows data for the first 45 minutes for six normal individuals, five men and one woman, whose weights varied from 50 to 75 kg. (No adjustment is

[1] A preliminary report.

[2] From the Departments of Radiological Research and Surgery, College of Physicians and Surgeons, Columbia University, with the aid of a grant from the Lilla Babbit Hyde Foundation.

[3] Hubbard, Preston and Ross have employed radiosodium in a somewhat similar manner to determine circulation time in infants, although the present authors were unaware of this when they commenced their study. See *Jour. Clinical Investigation*, 21: 613, 1942.

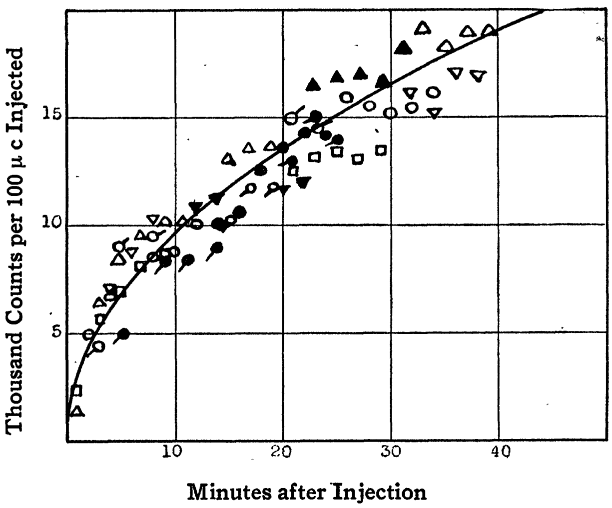

FIG. 1. Normal, six individuals. Counts at feet per 100 micro-curies of radio-sodium injected. Solid points, right foot; open points, left foot. Average curve drawn through experimental data.

made for variation in weight. Only alternate minutes are recorded, to prevent crowding the chart.) Solid points indicate counts for the right foot, open ones for the left, per 100 micro-curies of radio-sodium injected. All values fall within ±15 per cent. of an average curve. During the next hour the values increase by about 25 per cent., and in the following 24 hours pos-

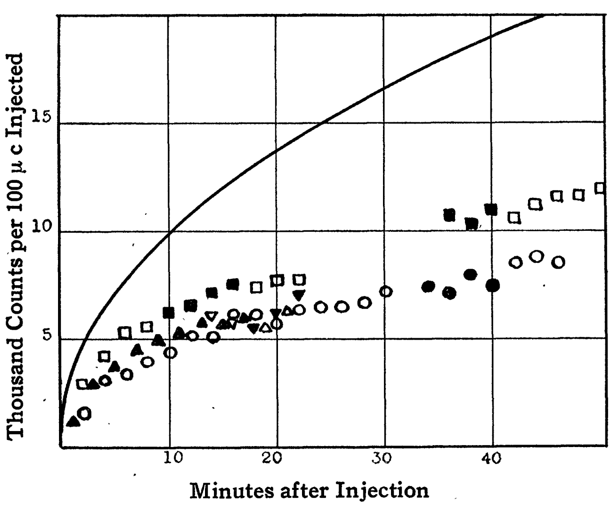

FIG. 2. Scleroderma and Raynaud's disease. Normal curve from Fig. 1; experimental points all well below it.

sibly 10 per cent. more (allowance, of course, being made for radioactive decay). Excretion in the first 24 hours is not more than 5 per cent., and probably less. Measurement on total urinary excretion in two normal individuals gave 2.5 per cent. of the injected radio-sodium in the first 18 hours.

In some cases of various types of peripheral vascular disease, as diagnosed clinically, the curves fell within the normal range. In others, however, there were marked differences. The thromboangitis obliterans cases all fell near the lower limits of the normals, as did most of the arteriosclerotics. Further study may lead to some way of differentiating among these. On the other hand those with diabetic gangrene, scleroderma and frostbite ("immersion foot") all fell well below the limits of the normal group. In Fig. 2 the heavy line shows the average normal curve; the points are for three cases of scleroderma and one of Raynaud's disease. This last (the squares) was rather surprising, for the patient's symptoms were confined to her hands. In Fig. 3, the heavy line is again the

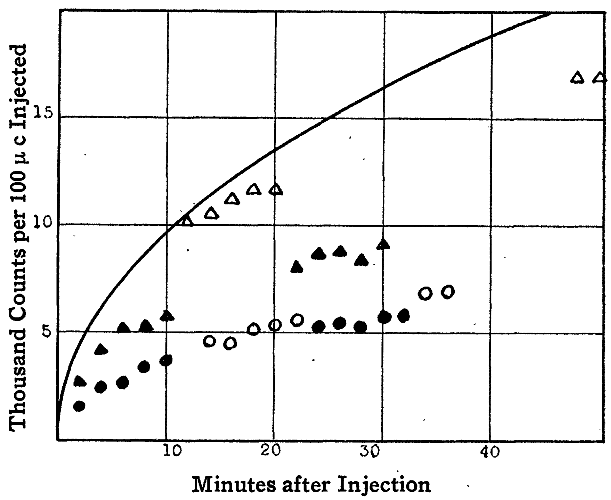

FIG. 3. "Immersion Foot." Normal curve from Fig. 1. Case indicated by circles had both feet affected, case indicated by triangles had left foot nearly normal.

normal curve; the points represent two cases of immersion foot. In the case indicated by the circles, the feet appeared normal, except that they were cold to the touch, and the patient had been certified as fit for active duty. He complained of pain on standing or walking and especially on being chilled; the feet were about equally affected. The case indicated by the triangles had a right foot much worse than the left. The solid points are for the right foot; the open ones for the left, which was almost normal. It appears that a test of this type would make possible the differentiation between cases which had recovered and those which had not. The study should be extended to the acute type of frostbite suffered by aviators in short exposures to very low temperatures, in contrast to the long exposures at not such low temperatures but with accompanying soaking endured by those drifting in open boats for several days.

Differential reactions between the two feet have been observed in a number of individuals with various complaints, and may be useful as a guide to treatment. In Fig. 4, the circles are data for a woman with dia-

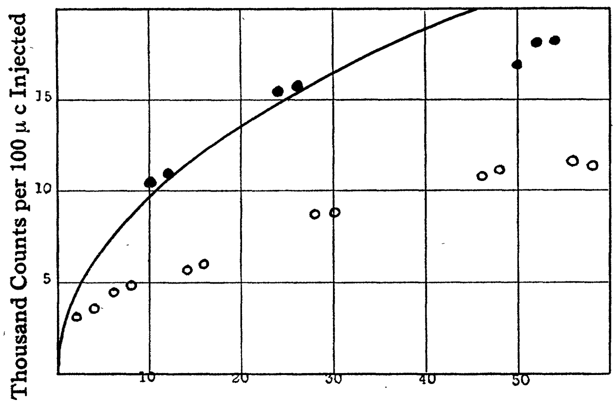

FIG. 4. Diabetes. Normal curve from Fig. 1. Right foot normal, left gangrenous. Subsequently amputated, with poor wound healing.

betic gangrene in the left foot, no symptoms in the right. The solid circles, for the right foot, fall within normal limits; the open ones for the left, are low. The left foot was subsequently amputated, with poor wound healing. In other cases the region of the leg at which normal counting occurred has been used satisfactorily as a guide to position of amputation.

The interpretation of the type of count which increases more slowly than normal, but eventually, even after several hours, reaches the normal level, is probably concerned with the condition of the circulatory system. In those cases in which the count does not come up to normal after several hours, the explanation may be different and may have to do with actual change in the so-called "sodium space" in the foot.

Sodium space has been determined by Kaltreiter and his associates and by others, by injecting radiosodium as in the present work and then withdrawing blood at a certain time subsequently, and determining the ratio of counts per cu cm of serum to counts for the total material injected, under standard conditions.[4] Further studies along this line are being carried out, for patients with vascular disease.

Up to the present time fifty individuals have been studied, some in more detail than others. In each disease group only a few cases are available; however, the data have already proved of value in diagnosis and prognosis, and it is evident that further work is desirable.

An application of the method which has not as yet been employed to any extent is as an evaluation of therapeutic procedures. If a patient is tested before treatment is instituted and at intervals while it is being carried out, any change either for the better or worse should be indicated. Repetitions of the test at intervals of a few weeks would be entirely safe, since the amount of radiation dose for each test is so small.

The authors wish to express their indebtedness to the Radiation Laboratory of the Department of Physics of Columbia University, for providing the Geiger-Müller counting apparatus and for the preparation of the radioactive sodium.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A DRY ICE FREEZING UNIT FOR ROTARY MICROTOMES

TISSUE sections cut from the frozen state are being utilized in an increasing number of techniques, but the equipment necessary for cutting such sections is not always available. The methods in common use require either a sliding microtome or a specially designed "freezing" microtome. However, the rotary microtome is the only kind available in many laboratories, and although it is generally considered to be unsuited for cutting frozen sections, it may be quite satisfactorily adapted for this purpose by the use of the device illustrated in Fig. 1.

This consists of a metal box made to conform to the shape of the object clamp of the microtome in which it is to be used, but about 2 mm smaller on all sides to allow for insulation. (The dimensions shown are suitable for No. 818 and No. 820 Spencer microtomes.) One end is made from a piece of sheet copper, to the outer surface of which is soldered a metal object holder about 2 mm in thickness and large enough to accommodate the desired specimens. The face of the object holder is grooved to provide for the firm attachment of the specimen and its entire back surface must be uniformly fused to the end of the box to allow for rapid and efficient heat transport. The sides of the box are made from a single sheet of copper bent to the same size and shape as the end and soldered to it. The other end is made of asbestos board, bakelite or other insulating material. It fits tightly into the open end of the box and should have a small handle to allow easy removal. The entire unit, except the surface of the object holder, is insulated by cementing on a layer of asbestos paper and then covering this with a layer of hard-surfaced paper and several coats of waterproof varnish.

[4] N. L. Kaltreiter, G. R. Meneely, J. R. Allen and W. F. Bale, *Jour. Exper. Medicine,* 74: 569, 1941.