tions, being practically quantitative at low salt concentrations, and progressively poorer at higher concentrations. For example, in 1.7% NaCl solution (i.e., twice isotonicity) recovery was only 85% (Table 2).

These results demonstrate that under appropriate conditions it is possible by these techniques to separate quantitatively the vitamin B_{12} from dilute solutions. If the vitamin B_{12} to be assayed contains Co^{60}, radioactivity in the cells may be taken as a measure of the vitamin content. This method is ideally suited to the estimation of radioactive vitamin B_{12} in the urine of rats given small subcutaneous injections. In this situation the samples could not be assayed satisfactorily by the usual microbiological method, both because of the low activity and because of the presence of inhibitors that prevented the use of larger aliquots. Approximate measurements could be made if the vitamin were separated by repeated extraction with normal butanol after the addition of solid ammonium sulfate to the specimens, and the radioactivity in the butanol determined. Results of determinations following both *L. leichmannii* uptake and the butanol extraction of radioactivity in urine collected 24 hr after subcutaneous injection of 1.0 μg of Co^{60} tagged vitamin B_{12} are compared in Table 3.

TABLE 3

DETERMINATION OF RADIOACTIVE VITAMIN B_{12} IN RAT URINE

Samples	Method employed*	
	Uptake by resting cells	Butanol extraction
Normal rat urine + 5 mμg vitamin B_{12}	4.2	3.5
Normal rat urine + 20 mμg vitamin B_{12}	18.5	14.0
Normal rat urine + 40 mμg vitamin B_{12}	37.0	26.0
Urine (*A*)†	9.0	6.0
Urine (*B*)†	12.0	7.8
Urine (*C*)†	13.6	10.2

* The radioactive vitamin B_{12} recovered by each method was expressed as mμg/sample (20 ml). One mμg was found to give 10 cpm.

† The samples of urine were obtained from adult rats 24 hr after injection of 1 μg radioactive vitamin B_{12}.

They demonstrate that the activity obtained by the method of absorption on microorganisms yielded consistently higher results than the extraction method. The higher values are probably more nearly correct, since control recovery experiments in which known amounts of radioactive vitamin B_{12} were added to normal urine gave more nearly quantitative results by the *L. leichmannii* uptake method.

The rapid and quantitative isolation from solution of vitamin B_{12} by microorganisms such as *L. leichmannii* also lends itself to assay of nonradioactive vitamin B_{12}. This has been accomplished either by the isotope dilution technique (i.e., by adding a known amount of radioactive vitamin B_{12} to the test solution followed by subsequent addition of a standardized suspension of organisms with uptake of a definite amount of vitamin B_{12}) or by microbiological determination of vitamin B_{12} in the cell mass. In the latter case, after centrifugation and washing, if the cells are suspended in normal saline solution and killed by heating at 60° for 1 hr, the vitamin B_{12} in the cell mass is available to the test organisms used in the assay method of Skeggs and Wright. The main advantage of our procedure lies in concentrating the small quantities of vitamin B_{12} by the use of bacteria. The details of these methods and further applications will be published elsewhere.

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Manuscript received January 3, 1952.

On the Interaction of Mesons and Plural vs. Multiple Meson Production

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Recent experimental work by Bernardini *et al.* may throw some light on the nature of the interaction of π-mesons with nucleons as well as suggest a new interpretation of the problem of multiple vs. plural meson production. It is well known that the question of multiple vs. plural meson production may be in good part an outgrowth of our lack of knowledge concerning the nature of nuclear forces and the elementary interaction process (*1*).

Bernardini, Booth, and Lederman (*2*) have found that the very strong inelastic nuclear scattering of negative π-mesons in which the energy losses exceed 50%, as well as the negative π-meson nuclear absorption involving catastrophic processes, are strongly energy-dependent and occur only for energies greater than about 60 mev. The frequency of these anomalous scatterings, as well as the existence of cases showing energy losses close to 100%, casts doubt on the validity of the free nuclear model of the nucleus (*3*). The possibility of explaining the observations in terms of multiple elastic collisions inside the nucleus, instead of the single π-nucleon collisions, is also unlikely in view of the recent results of Chedester *et al.* (*4*). In a personal communication Bernardini has informed the writer that there is some direct evidence that this type of anomalous scattering could be interpreted as an interaction of a negative π-meson with a group of nucleons (containing 3 or 4 nucleons) acting as a *whole*. The scattering experiments cited above suggest the excitation of such nuclear groups or subunits of the nucleus, and may indicate the operation of a many-body type process. The experimental results also lend themselves to an interpretation which suggests that the anomalous scattering of π-mesons of energies greater than about 60 mev by complex nuclei

may be a function of the density of the nucleons in a volume of the order involving the Compton wavelength of the meson.

There is other recent evidence of the failure of conventional interactions and models. In particular, one may cite the work of Chamberlain, Segrè, and Wiegand (*5*) on *p-p* scattering from 120 to 345 mev. These authors have found that all theoretical investigations so far, based on static potentials, including the work of Christian, Jastrow, and others, have failed to explain their experimental results. Thus, the experimental evidence of both Bernardini *et al.* and Chamberlain *et al.* points to the possible operation of nonlocalizable, velocity-dependent forces of the many-body type.

Osborne (*6*) has presented experimental evidence of pluromultiple meson production in high-energy cosmic-ray showers. He points out that, although the events in light nuclei are not inconsistent with a single collision model, the heavier nuclei events cannot be described by this model. In view of the probable nature of nuclear forces as indicated by the evidence from the scattering experiments cited above, it is the author's opinion that the simple picture of pure plural meson production by individual nucleon-nucleon encounters is quite likely not valid. What in the past have been taken for examples of pure plural meson production in cosmic-ray showers by individual nucleon-nucleon encounters probably involve multiple production in one single act by nucleons acting as nuclear subunits or groups. These groups of nucleons may be those outside the nuclear core, which is consistent with evidence presented by R. F. Mozley (*7*) and R. D. Miller (*8*) on photonuclear stars, which indicates that only the surface nucleons are effective in producing π-mesons. In the phenomenon of pluromultiple production there may be more than one such group involved. Although it is not unlikely that there may be no such phenomenon as pure plural meson production, there seem to be two types of multiple meson production: the type ordinarily encountered involving nucleonic groups and the type first observed by Lord, Fainberg, and Schein (*9*), wherein the nucleonic volume involved in these extremely high-energy interactions is so small as to preclude the presence of groups of nucleons, but nevertheless may be the site of the excitation of the nucleon-antinucleon field, the heavy nuclear quanta fields (embracing mesons of the τ and V type) and the surrounding pion field.

The volumes of interaction discussed above suggesting the nonlocalizability of nucleonic interactions may be termed "elementary volumes" and may be identified as functions of the elementary lengths. Thus, there is more than one elementary length, and in this context they may be interpreted as not so much imposing limitations to the behavior of natural phenomena as the natural constants c, e, and h do, but rather as serving to define the application of various physical models and concepts. For instance, in the experiments of Bernardini *et al.* the elementary length involved (the mesonic Compton wavelength) limits the application of the free nucleon model of the nucleus, as well as the concept of the static potential (*10*). On the other hand, the elementary length (probably the protonic Compton wavelength) involved in the phenomenon of the multiple production of particles involving single nucleon volumes may limit, if one is permitted to speculate, the unambiguous application of the elementary concepts of particle and field.

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Manuscript received November 19, 1951.

A Rapid Titrimetric Method for Determining the Water Content of Animal Tissues[1]

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The most widely used method for determining the water content of animal tissues is that whereby the material is dried to a constant weight in an oven. This method is subject to certain disadvantages, notably the danger of decomposing some of the complex organic compounds if too high a temperature is employed and the time consumed in drying and weighing. These difficulties are to a great extent obviated by the use of the Karl Fischer reagent and the titrimetric estimation of water.

This procedure is fully described in the recent book by Mitchell and Smith (*1*) and has been widely utilized in industrial laboratories for the determination of the water content of many substances such as paper, reagents, and foodstuffs. We have encountered no record of its adoption by physiologists and biochemists, but we believe that it would prove of considerable value to these investigators.

For determining the water content of blood we first weigh a capillary tube approximately ½ in. in length.

[1] This investigation was carried out under contract N7onr29544 between the Office of Naval Research and the University of California.

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