at 7 weeks of age is about 56 per cent. A rat of similar age having been on a rachitogenic (Steenbock) diet for 3 weeks has a femur ash which varies somewhat with rat strain, season, etc. In the experiments reported here the mean value was 30 per cent., ranging from 24 to 34 per cent. When ample Vitamin D was given during the three weeks on the rickets-producing diet, the bone ash did not rise above a mean of 50 per cent. (49 to 52 per cent. in groups of about 10 animals); with added phosphate, however, the bone ash values clustered very closely about the normal 56 per cent. The 50 per cent. bone ash level was attained with 0.5 U.S.P. unit of D per day. Several times higher Vitamin D intake produced no further increase. Dose response curves over a significant range have been worked out.

Ten-day curative experiments carried out as Vitamin D assays (U.S.P. technique) gave the following results. A definite fairly wide continuous line of recalcification (++ on our scale) raised the bone ash to no higher than a mean value of 34 per cent., with about the same variation as applied to the 30 per cent. value for negative controls. Effects of this magnitude are commonly produced by 0.2 to 0.3 units of D per day. Distinctly visible recalcification but less than the ++ healing gave extremely variable bone ash values and very poor correlation with the visual picture. In several groups no increase whatever was shown in bone ash when mean values were compared. Fifty or a hundred times the dose which produced a ++ healing did not raise the bone ash above 40 per cent., although the silver stained bone showed the entire previously rachitic area filled with dense newly formed calcified trabeculae.

It is indicated therefore that in curative experiments a definite effect noted visually may correspond to but a small fraction of the healing process, and that what is considered very substantial healing effects raise the bone ash through only about one fifth of the interval between the rachitic and the full Vitamin D prevention levels. One hundred times this dose raises the bone ash half way towards the prevention level. Any further increase requires more time.

Besides these planned experiments, observations on more than 400 bones from assay studies, mostly on cod liver oil concentrates but also including other forms of Vitamin D, verify this picture. It is not surprising to find that the variation in bone ash values increases, the further they are removed from normal.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

PREPARATION OF METHIONINE-FREE NATURAL LEUCINE

IN a recent note in SCIENCE, Mueller[1] pointed out that commercial l-leucine is contaminated with methionine. The presence of a sulfur-containing impurity was recognized by Emil Fischer[2] in 1900. Unsuccessful attempts to purify natural l-leucine have included: the amino-acid ester method, repeated recrystallization from water, electrodialysis, precipitation of methionine as mercury complex and recrystallization from acidified butyl alcohol. Recrystallization of the formylated amino-acid has now been found, however, to yield a sulfur-free leucine without appreciable racemization.

Mixtures of l-leucine samples containing 2.6, 5.5 and 8.0 per cent. methionine were formylated by the methods of Fischer and Warburg[3] and of Steiger.[4] Two recrystallizations from water rendered the formyl-leucine practically sulfur-free, as determined by combustion sulfur analysis. For purposes of precise calorimetry, these samples were recrystallized from water four more times. The formyl-leucine was then decomposed by refluxing one hour with ten cc of 10 per cent. HBr per gm of formyl-leucine, and most of the excess acid removed by evaporation *in vacuo*. After solution of the residue in water, the evaporation was twice repeated. The final residue was dissolved in a little water, neutralized with conc. NH_4OH, chilled, and the crystals were filtered off. The leucine was washed with alcohol until halide-free, and finally recrystallized from water.

The yield of leucine from original crude material, after six recrystallizations of formyl body, was 50 per cent. The product was sulfur-free and ash-free. The specific rotation, in 20 per cent. HCl, checked that of l-leucine obtained by synthesis and resolution.

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1 J. H. Mueller, SCIENCE, 81: 50–51, 1935.
2 E. Fischer, *Ber. chem. Ges.*, 33: 2372, 1900.
3 E. Fischer and O. Warburg, *Ber. chem. Ges.*, 38: 3998, 1906.
4 R. E. Steiger, *Jour. Biol. Chem.*, 86: 695, 1930.

A DIFFERENT PRINCIPLE IN THE CONTINUOUS RENEWAL OF CULTURE SOLUTION

DURING the past fifteen years numerous pieces of apparatus have appeared that were planned to deliver culture solutions at a uniform rate. Several of these employ the principle of dripping from a nearly constant level in order that the rate of drip be uniform.

While conducting solution culture experiments during last year, the author found that by the principle of maintaining the dripping unit at the changing level of the solution in the reservoir of fresh solution, the problem was simplified. A most uniform, continuous flow may be accomplished in this way. Fig. 1 shows the construction of the apparatus employed in this work.

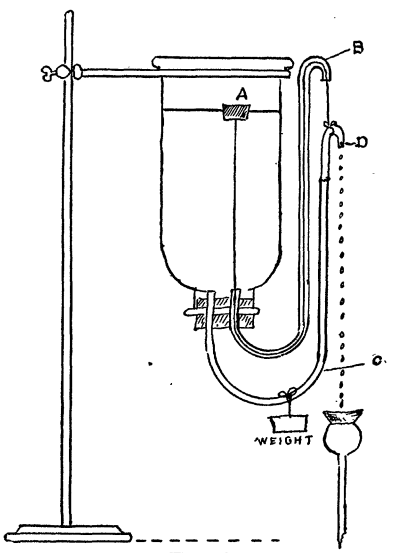

FIG. 1

In this apparatus a piece of paraffined twine passes from the cork float *a* to the bottom of the reservoir, then through a stationary tube *b*, and is fastened to the dripping unit *d*. The dripping unit *d* descends at the same rate as the level of the solution in the reservoir, since it takes up the slack of the cord which leads from the float *a*. The descent of the dripping unit is made possible by a rubber tube connection *c*, bearing a small weight.

This apparatus combines simplicity of construction with a readily adjustable flow of solution. Neither capillary tubing nor clamps are required in its construction. Air bubbles are carried through a thistle tube[1] into the culture jar with the drops of solution, thereby providing a constant oxygen supply for the plant roots.

The author wishes to express appreciation to Mr. C. W. Myers for making the diagram in Fig. 1.

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A CONVENIENT RETRACTOR FOR USE IN OPERATIONS AND DISSECTIONS OF SMALL-SIZED ANIMALS

THE following instrument is recommended for use as holder or retractor in operations and other manipulations on small objects: Into the top of a rubber vacuum suction cup (about 1 inch in diameter) a glass rod is fitted. The rod is bent into a horizontal position, drawn out to a suitable width, and its end is shaped into a hook, as shown in the diagram. The

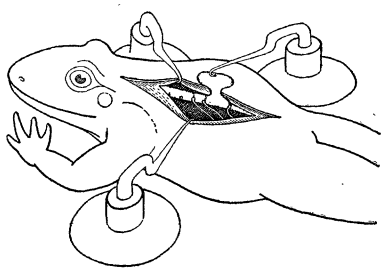

FIG. 1

moistened cup, when pressed against the polished surface (glass, enamel, bakelite, etc.) on which the animal rests, sticks for any length of time, giving the hook a firm hold, with enough elasticity to prevent injury to the retracted tissues. Horizontal adjustments are effected by sliding the cup along the table surface. Vertical adjustments within narrow limits can be obtained by pressing the cup more or less tightly against the table. If vertical adjustments of greater extent are desired, the glass hook should be mounted on a metal cuff sliding up and down a metal rod inserted into the top opening of the rubber cup. Skin edges, muscles, blood vessels, nerves, etc., can thus be conveniently gripped and retracted so as to give free vision and access into the deeper fields of the operation.

With appropriate modifications the instrument can be used for many other purposes in the laboratory, for instance, as holder for stimulating electrodes.

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[1] R. P. Marsh, SCIENCE, 82: 256, 1935.

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