distal to the partly occluded pylorus. In one dog a perforated ulcer at this point, with death from peritonitis, was found.

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Concentration of Calcium Ions in Biological Fluids.

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The sensitivity of the frog's heart to changes in the calcium content of a nutrient fluid has long been known[1, 2]. Utilization of this property for the estimation of calcium ions in biological fluids has, however, not been generally considered feasible, because considerable doubt has existed[3] as to whether the known sensitivity is to calcium ions or to total calcium. The method here presented is based upon (1) the demonstration, simultaneously reported, that the reaction of the frog's heart is specific for calcium ions, bound calcium being without effect, and (2) elimination or control of other factors which may influence the behavior of the heart.

The method consists essentially of recording the amplitude of the contraction of the frog's heart, suspended from a modified Straub cannula inserted through the aorta into the ventricle. After taking certain precautions to standardize the procedure, the concentration of calcium ions in the unknown solution or biological fluid is taken to be that of the known solution which produces an amplitude of contraction most nearly matching that produced by the unknown. A satisfactory preparation is sensitive to changes of 0.1 millimol per liter of calcium ions (0.4 mg. per 100 cc.) or less, so that the theoretical accuracy of the method is to within $\pm$0.1 mM per liter.

The method is applicable to such solutions or biological fluids as will sustain the action of the frog's heart without toxic effects. It can be used in artificially prepared salt solutions of widely varying composition, and has been found suitable for serum, plasma, cere-

[1] Straub, W., *Verhandl. d. Gesellschaft deutscher Naturforscher und Aerzte*, 1912, **84**, 192.

[2] Trendelenburg, P., und Goebel, W., *Arch. f. exp. Path. u. Pharm*, 1921, **89**, 171.

[3] Klinke, K., *Ergebnisse der Physiol.*, 1928, **26**, 235,

brospinal fluid, chest fluid, and ascitic fluid of human origin, and for serum and cerebrospinal fluid of the dog. It has been found unnecessary to dilute these fluids, the only precaution being to make the known solutions isotonic with the unknowns, rather than with frog's blood.

The solutions used for comparison have been based upon the solution of van Dyke and Hastings[4] and made up to resemble as closely as possible the inorganic constituents of the fluids to be matched. It has been shown that with the frog's heart as indicator, variations of temperature, pH, magnesium, and phosphates within a rather wide range have little or no effect, and that a change of 1.0 mM in potassium concentration produces at the most less effect than a change of 0.1 mM in calcium concentration, so that it has been found possible to make up standard solutions approximating the average inorganic composition of the biological fluids to be studied, and in fact to omit magnesium and phosphates entirely from these standard solutions.

The only real difficulty with respect to biological fluids, and a possible source of error, is with respect to the presence or possible presence of pressor or depressor substances in these fluids, which may conceivably either mimic or mask the effect of calcium ions present in the fluid. Many of the biological fluids so far examined have contained a substance or substances increasing the amplitude of contraction of the heart, particularly if the heart is in a "hypodynamic" state. With fresh hearts this effect may not be observed, except when dog serum is used, this serum being rich in pressor substance.

In practice it is readily observed that the effects of pressor substances may easily be distinguished from the effects of calcium ions, chiefly by the rate of response of the heart to them, and by the rate of recovery after they are withdrawn. The response to changes in calcium ion concentration is an immediate one, the heart frequently being in equilibrium with a new solution by the time the change is completed. The effect of a pressor substance, however, is much more gradual, and persists for a much longer time after it is withdrawn. In practice, therefore, when the effect of a pressor substance is observed it is allowed to produce its maximum effect, and comparisons are then made at the new level just as though no pressor substances were present. That this method is valid has been shown by experiments in which adrenalin has been added to known solutions.

[4] van Dyke, H. B., and Hastings, A. B., *Am. J. Physiol.*, 1928, **83**, 563.

Thus *recognizable* pressor effects have been eliminated as a complicating factor. We have not, however, up to the present, eliminated the possibility of other pressor or depressor substances having a speed of action and disappearance comparable to that of calcium ions. On the other hand, we have encountered no instances suggesting the presence of such substances.

Using the method as described the calcium ion concentration in certain biological fluids has been estimated. (Table 1).

TABLE I.
Estimations of Ca++ Concentration in Biological Fluids.

Fluid	Source	Diagnosis	Total Ca mM/L	Ca++ mM/L
C.S.F.	Human	Epilepsy	1.37	1.15
Serum	"	Normal	—	1.0
C.S.F.	"	No diagnosis	1.24	1.0
C.S.F.	Dog	Tetany	1.04	0.95
Serum	"	Normal	2.36	0.9
Chest	Human	Heart failure	2.11	0.8
Ascitic*	"	Pick's disease	3.00	1.05

*High protein content.

The concentration of calcium ions in the fluids studied was approximately 1.0 millimol per liter. From this, the non-ionized, diffusible calcium is estimated at not more than 0.25 millimol per liter in cerebrospinal fluid and, after allowance is made for the calcium bound by protein, not more than 0.5 millimol per liter in serum.

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Electrical and Mechanical Changes in Isolated Heart Following Changes in Calcium Content of Perfusing Fluid.

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The experiments here reported were performed on the isolated heart of the rabbit perfused at 38°C. by the method described by Locke, but without recirculation of fluids. Changes in amplitude of contraction were recorded on a drum, and electrical changes were recorded with a string galvanometer from one circuit only, the connections being made through non-polarizable electrodes to the aorta and to the apex.