The examination (in which I was aided by Doctors Maximow and Cannon, of the University of Chicago) revealed that there were no intracellular infiltrations, no casts or blood in the tubules, and both the glomeruli and tubules appeared to be normal. The liver showed no trace of necrosis, and differed in no respect from that of a normal dog.

CONCLUSIONS.

Ceanothyn, administered orally to dogs in doses thirty to forty times the daily human dose, over a period of seventeen days, produced no detectable impairment of the kidneys and liver in the dog as shown by functional tests and morphological studies.

HYDROGEN-ION CONCENTRATION AND p_H.

(COLORIMETRIC DETERMINATIONS. PAPER II.)

BY A. LEE CALDWELL.

There are so many articles on making hydrogen-ion or p_H determinations that one hesitates to add to that number. However, each writer has his personal claims to merits of the methods that he advocates. Perhaps it should be said that there is, primarily, but one colorimetric method, and that this one method has many modifications, the entire success of which depends upon the reactions of certain indicators with acid and alkaline solutions. A great many forward steps have been taken in the development of indicators which show two distinct colors and all the variations between the two colors, dependent of course upon the hydrogen-ion concentration for the change of color. It will not be necessary here to go into detail regarding the theory of indicator properties. Even if it were attempted there could be nothing more than a general or "planket" decision made as to the why and wherefore of indicator action. Ostwald, who proposed the theory that molecular rearrangement was the cause of the color change, has not been disproven, although many other investigations have revealed additional causes.

The old and useful indicators of vegetable and animal origin, such as litmus and cochineal, are rapidly being replaced in the fields of acidimetry and alkalimetry, for many indicators react more easily to small amounts of acid or alkali and their uses are varied. Litmus is still useful but hardly has a place in control work of chemicals and pharmaceuticals. The most widely used indicators and the best for work in all fields are the sulphon-phthalein indicators, and they are the ones which will be discussed in this paper.

It might be well to emphasize the need of using indicators of reliable make and the necessity of checking the determinations with at least two indicators. Necessary to the determination of the p_H are standard buffer solutions with which the unknown solutions may be compared. Buffer solutions are solutions of acids or alkalies together with salts of these acids or alkalies, in most cases, sufficient to give a concentration producing the desired p_H and to withstand the effects of small amounts of acid or alkali with which they may come in contact. The most generally used and easily prepared buffers are those of Clark and Lubs (*J. Biol. Chem.*, 25 (1916), 479). They are prepared as follows:

p_H.			
1.2	50 cc. $M/5$ KCl	64.5 cc. $M/5$ HCl	Dilute to 200 cc.
1.4	50 cc. $M/5$ KCl	41.5 cc. $M/5$ HCl	Dilute to 200 cc.
1.6	50 cc. $M/5$ KCl	26.3 cc. $M/5$ HCl	Dilute to 200 cc.
1.8	50 cc. $M/5$ KCl	16.6 cc. $M/5$ HCl	Dilute to 200 cc.
2.0	50 cc. $M/5$ KCl	10.6 cc. $M/5$ HCl	Dilute to 200 cc.
2.2	50 cc. $M/5$ KCl	6.7 cc. $M/5$ HCl	Dilute to 200 cc.
2.2	50 cc. $M/5$ KH Phthalate	46.70 cc. $M/5$ HCl	Dilute to 200 cc.
2.4	50 cc. $M/5$ KH Phthalate	39.60 cc. $M/5$ HCl	Dilute to 200 cc.
2.6	50 cc. $M/5$ KH Phthalate	32.95 cc. $M/5$ HCl	Dilute to 200 cc.
2.8	50 cc. $M/5$ KH Phthalate	26.42 cc. $M/5$ HCl	Dilute to 200 cc.
3.0	50 cc. $M/5$ KH Phthalate	20.32 cc. $M/5$ HCl	Dilute to 200 cc.
3.2	50 cc. $M/5$ KH Phthalate	14.70 cc. $M/5$ HCl	Dilute to 200 cc.
3.4	50 cc. $M/5$ KH Phthalate	9.90 cc. $M/5$ HCl	Dilute to 200 cc.
3.6	50 cc. $M/5$ KH Phthalate	5.97 cc. $M/5$ HCl	Dilute to 200 cc.
3.8	50 cc. $M/5$ KH Phthalate	2.63 cc. $M/5$ HCl	Dilute to 200 cc.
4.0	50 cc. $M/5$ KH Phthalate	0.40 cc. $M/5$ NaOH	Dilute to 200 cc.
4.2	50 cc. $M/5$ KH Phthalate	3.70 cc. $M/5$ NaOH	Dilute to 200 cc.
4.4	50 cc. $M/5$ KH Phthalate	7.50 cc. $M/5$ NaOH	Dilute to 200 cc.
4.6	50 cc. $M/5$ KH Phthalate	12.15 cc. $M/5$ NaOH	Dilute to 200 cc.
4.8	50 cc. $M/5$ KH Phthalate	17.70 cc. $M/5$ NaOH	Dilute to 200 cc.
5.0	50 cc. $M/5$ KH Phthalate	23.85 cc. $M/5$ NaOH	Dilute to 200 cc.
5.2	50 cc. $M/5$ KH Phthalate	29.95 cc. $M/5$ NaOH	Dilute to 200 cc.
5.4	50 cc. $M/5$ KH Phthalate	35.45 cc. $M/5$ NaOH	Dilute to 200 cc.
5.6	50 cc. $M/5$ KH Phthalate	39.85 cc. $M/5$ NaOH	Dilute to 200 cc.
5.8	50 cc. $M/5$ KH Phthalate	43.00 cc. $M/5$ NaOH	Dilute to 200 cc.
6.0	50 cc. $M/5$ KH Phthalate	45.45 cc. $M/5$ NaOH	Dilute to 200 cc.
6.2	50 cc. $M/5$ KH Phthalate	47.00 cc. $M/5$ NaOH	Dilute to 200 cc.
5.8	50 cc. $M/5$ KH_2PO_4	3.72 cc. $M/5$ NaOH	Dilute to 200 cc.
6.0	50 cc. $M/5$ KH_2PO_4	5.70 cc. $M/5$ NaOH	Dilute to 200 cc.
6.2	50 cc. $M/5$ KH_2PO_4	8.60 cc. $M/5$ NaOH	Dilute to 200 cc.
6.4	50 cc. $M/5$ KH_2PO_4	12.60 cc. $M/5$ NaOH	Dilute to 200 cc.
6.6	50 cc. $M/5$ KH_2PO_4	17.80 cc. $M/5$ NaOH	Dilute to 200 cc.
6.8	50 cc. $M/5$ KH_2PO_4	23.65 cc. $M/5$ NaOH	Dilute to 200 cc.
7.0	50 cc. $M/5$ KH_2PO_4	29.63 cc. $M/5$ NaOH	Dilute to 200 cc.
7.2	50 cc. $M/5$ KH_2PO_4	35.00 cc. $M/5$ NaOH	Dilute to 200 cc.
7.4	50 cc. $M/5$ KH_2PO_4	39.50 cc. $M/5$ NaOH	Dilute to 200 cc.
7.6	50 cc. $M/5$ KH_2PO_4	42.80 cc. $M/5$ NaOH	Dilute to 200 cc.
7.8	50 cc. $M/5$ KH_2PO_4	45.20 cc. $M/5$ NaOH	Dilute to 200 cc.
8.0	50 cc. $M/5$ KH_2PO_4	46.80 cc. $M/5$ NaOH	Dilute to 200 cc.
7.8	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	2.61 cc. $M/5$ NaOH	Dilute to 200 cc.
8.0	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	3.97 cc. $M/5$ NaOH	Dilute to 200 cc.
8.2	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	5.90 cc. $M/5$ NaOH	Dilute to 200 cc.
8.4	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	8.50 cc. $M/5$ NaOH	Dilute to 200 cc.
8.6	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	12.00 cc. $M/5$ NaOH	Dilute to 200 cc.
8.8	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	16.30 cc. $M/5$ NaOH	Dilute to 200 cc.
9.0	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	21.30 cc. $M/5$ NaOH	Dilute to 200 cc.
9.2	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	26.70 cc. $M/5$ NaOH	Dilute to 200 cc.
9.4	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	32.00 cc. $M/5$ NaOH	Dilute to 200 cc.
9.6	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	36.85 cc. $M/5$ NaOH	Dilute to 200 cc.
9.8	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	40.80 cc. $M/5$ NaOH	Dilute to 200 cc.
10.0	50 cc. $M/5$ H_3BO_3, $M/5$ KCl	43.90 cc. $M/5$ NaOH	Dilute to 200 cc.

Large quantities of the fifth molar solutions may be made up at one time and may serve as convenient stock solutions to have ready when it is desired

to replace or replenish the more dilute buffer solutions. Redistilled water should be used unless the water was at first distilled slowly from a very good still supplied with steam under pressure. The salt should be recrystallized at least three times and the sodium hydroxide should be prepared by dissolving it in an equal weight of water in a pyrex flask and allowing it to stand until the carbonate settles out. This solution should be filtered through glass wool with suction and diluted with previously boiled distilled water to approximately *M/5* and a small quantity of this solution titrated. It is more convenient to use a factor than to make an exactly *M/5* solution. The hydrochloric acid *M/5* may be prepared by diluting a reagent quality HCl to *M/5* and determining the factor by precipitation with silver chloride. The salts should be dried before using at the temperatures here given.

	Dry at.	For *M/5* sol.
Acid potassium phthalate	110–115° C.	40.83 Gm. per L.
Potassium chloride	120° C.	14.91 Gm. per L.
Acid potassium phosphate	110–115° C.	27.23 Gm. per L.

Boric acid dry in desiccator over $CaCl_2$. (For boric acid *M/5*, potassium chloride *M/5* use boric acid 12.40 Gm. and potassium chloride 14.91 Gm. per L.)

All stock solutions as well as the standard buffer solutions should be protected from CO_2 and air and from alkali by using pyrex or non-sol glassware.

The indicator solutions consists of 0.04% solutions of the indicator. Sodium hydroxide is used in making up these solutions in order to give the alkali salt of the indicator which is adjudged the most reliable form by Clark and Lubs. Following are the sulphon-phthalein indicators:

		p_H.
Thymol-sulphon-phthalein	Thymol blue	1.2–2.8 8.2–9.8
Tetra-brom-phenol-sulphon-phthalein	Brom phenol blue	3.0–4.6
Tetra-brom-*m*-cresol-sulphon-phthalein	Brom cresol green	3.8–5.4
Di-chlor-phenol-sulphon-phthalein	Chlor phenol red	5.0–6.6
Di-brom-cresol-sulphon-phthalein	Brom cresol purple	5.4–7.0
Di-brom-thymol-sulphon-phthalein	Brom thymol blue	6.0–7.6
Phenol-sulphon-phthalein	Phenol red	6.6–8.2
Ortho-cresol-sulphon-phthalein	Cresol red	7.2–8.8
m-Cresol-sulphon-phthalein	*m*-Cresol purple	7.4–9.0

These are the indicators that appear in "The Determination of Hydrogen Ions" 2nd edition, by Wm. M. Clark (see also *U. S. Public Health Reports* (Dec. 1926), 3051–75). Although other very useful indicators have been synthesized, these are the principal ones.

METHOD OF PREPARING INDICATOR SOLUTIONS.

Thymol blue	20 mg.	*N*/50 NaOH	2.15 cc.	Water to make 50 cc.
Brom phenol blue	20 mg.	*N*/50 NaOH	1.50 cc.	Water to make 50 cc.
Brom cresol green	20 mg.	*N*/50 NaOH	1.40 cc.	Water to make 50 cc.
Chlor phenol red	20 mg.	*N*/50 NaOH	2.35 cc.	Water to make 50 cc.
Brom cresol purple	20 mg.	*N*/50 NaOH	1.85 cc.	Water to make 50 cc.
Brom thymol blue	20 mg.	*N*/50 NaOH	1.60 cc.	Water to make 50 cc.
Phenol red	20 mg.	*N*/50 NaOH	2.85 cc.	Water to make 50 cc.
Cresol red	20 mg.	*N*/50 NaOH	2.65 cc.	Water to make 50 cc.
Meta cresol purple	20 mg.	*N*/50 NaOH	2.65 cc.	Water to make 50 cc.

The writer has kept the standard solutions as well as the indicator solutions in pyrex flasks fitted with rubber stoppers holding a delivery tube as illustrated, Fig. 1. It is necessary to keep the delivery tube stoppered to prevent exposure of the solutions to air and laboratory fumes. After removing the small stopper from the delivery tube a slight pressure on the rubber stopper will fill the tube.

METHOD OF DETERMINATION.

A convenient quantity of the unknown solution is taken in a test-tube and to this is added the desired amount of indicator solution. Clark suggests five drops of indicator to 10 cc. of solution, which is empirical in that the drops used by different workers will vary in size. This, however, is no criticism for it is understood

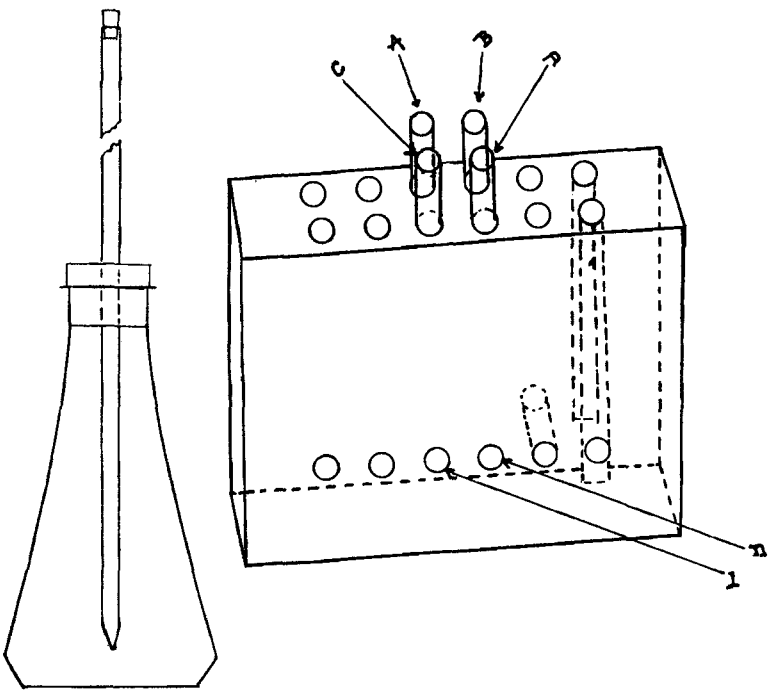

Fig. 1.—Flask on left. Fig. 2.—Block on right.

that each person may vary the amount of indicator used to suit the particular desire as to depth of color, etc. The writer preferred to use small test-tubes 0.7 cm. in diameter with 0.05 cm. wall and 8 cm. in length. These tubes have a volume of about 2.5 cc. and were graduated at 1 cc. Using 1 cc. in a tube of this type and using two drops indicator from a tube of small bore has given very good results with the conservation of a great deal of material and buffer solutions.

After adding the indicator the unknown solution is compared with several buffer solutions plus indicator, which have been taken in the same quantity as the unknown. The known p_H of the buffer solution which gives the same color as the unknown is taken as the expression of the p_H of the unknown.

Comparisons of color should be made under the same conditions for standard and unknown. Viewing the solutions by direct daylight is no doubt the best

method. Care must be taken to observe the depth of color as well as the shade obtained. As the limit in p_H range of an indicator is approached it is necessary to check the results obtained with other indicators to be sure that the correct p_H has been determined.

The p_H of cloudy or colored solutions may be determined using a block similar to the one illustrated in Fig. 2. Very good results have been obtained with solutions as dark as that given by iron cacodylate as well as with some "milky" solutions. One must be cautious to be sure that the determinations are correct, since in many cases with colored solutions the accuracy is not as great as with colorless solutions. Place a tube containing the unknown solution in the block (Fig. 2) in Position A; in Position B place a tube of distilled water; in Position D place a tube containing the unknown to which the indicator has been added, and C will represent the standard solution which will be changed until the color given matches that of Tube D when observed through holes l and n. When the color is matched the standard solution used in Tube C represents the p_H of the unknown.

The writer has used an aluminum block in which holes were bored of a size to hold the tubes loosely and deep enough to allow the curved bottom of the tubes to be below the lower side of the observation holes. These observation holes were bored in line with the holes for the tubes and just sufficiently smaller as to prevent looking at any time through the side walls of the tubes. Care should be taken that the salt concentration in unknown solutions is not misleading. It will be noticed in some cases that the shades produced are not at all comparable with the buffer solutions and may be accounted for by the fact that the salt concentration is not the same as in the standard buffer solutions, or that the solutions under examination contain a salt tending to destroy the indicator.

It is well to observe the depth or quality of the color produced; for example glucose solutions in some cases show the same shade as the standard buffers but do not seem to produce the same depth of color. Quinine dihydrochloride, quinine and urea hydrochloride and other quinine salts destroy the action of brom phenol blue entirely. Proteins as a class seem to have the greatest power for destroying the normal action of indicators. Pituitary extract is foremost among the gland extracts that destroy the indicator reactions, in fact it is not advisable to use the colorimetric method on concentrated extracts of any gland product.

It may be well to recommend an indicator suggested in Clark's "Determination of Hydrogen Ions," 2nd edition, composed of equal parts of methyl red indicator solution and brom thymol blue indicator solution and having a range of p_H 4.6 to 7.6. Experience will develop the technique and the ability to come very close in the p_H measurements. It is much better to use small tubes and to use freshly prepared color standards for comparison than to attempt to prepare a set of color standards for permanent use or even for only several days' use.

The following list of chemicals, will give an idea of how the p_H runs in C. P. chemicals purchased from various sources. There is a wide variation in some cases, the worst offender being C. P. sodium iodide.

Distilled water obtained from ordinary stills will range in p_H from 4.8 to 6.0, while stills carefully operated under the best conditions will produce water having a p_H of from 6.5 to 6.8 which is very good. Carbon dioxide is usually the principal

constituent that causes the low p_H in distilled water. The methyl red brom-thymol-blue indicator mentioned has given very good results with distilled water.

	p_H.	Indicator.
Caffeine	4.75–5.00–5.50–6.60	B. C. G.-B. T. B.-B. C. P.
Calcium chloride	7.45–7.90–8.10–8.90	P. R.-T. B.
Calcium glycerophosphate	8.20–8.30–8.35–8.40	P. R.-T. B.
Calcium lactate	6.85–7.00–7.50–7.90	P. R.
Dextrose	5.10–5.30–5.40–5.70	B. C. G.-B. C. P.
Hexamethylene-tetramine	7.70–8.00–8.20–8.40	P. R.-T. B.
Iron and ammonium citrate	3.10–3.50–4.00–4.60	B. P. B.-B. C. G.
Iron cacodylate	4.60–5.40–5.60–5.80	B. C. P.-B. C. G.
Magnesium sulphate	6.10–6.30–6.50–6.70	B. T. B. M. R. B. T. B.
Procaine	5.10–5.20–5.30–5.80	B. C. G.-B. T. B.
Potassium guaiacol sulphonate	7.45–7.60–7.70–7.90	P. R.
Sodium benzoate	7.70–8.20–8.50–9.30	P. R.-T. B.
Sodium cacodylate	7.85–8.00–8.30–8.45	P. R.-T. B.
Sodium citrate	7.70–8.00–8.30–8.40	P. R.-T. B.
Sodium glycerophosphate	8.60–8.70–8.90–9.00	T. B.
Sodium iodide	4.90–5.40–8.80–9.60	B. C. G.-T. B.
Sodium salicylate	6.90–7.05–7.10–7.40	P. R.-B. T. B.

For the p_H range 2.9 to 4.0 where bromo-phenol-blue has failed to give results dimethyl-amido-azobenzene has been used successfully. Under the title "A Universal Indicator for Hydrogen-Ion Concentration," Dr. Emil Bogen, in the *J. A. M. A.*, Vol. 89, 199, has suggested a mixture of phenolphthalein, 100 mg.; methyl red 200 mg; dimethyl-amino-azobenzene, 300 mg; brom-thymol-blue, 400 mg.; thymol blue 500 mg. Dissolve in 500 cc. of absolute alcohol and add tenth normal sodium hydroxide until the red disappears and the solution becomes yellow. This indicator will cover the p_H range from 1.0 to 10.0 and is very good to get the first estimate of the p_H; however the writer has found that economy may be had by using half the quantities of indicators and half the alcohol, and after solution adding water to make up the volume.

Samples of soils and similar materials may be soaked in a small amount of distilled water and the p_H determined on the filtered extract. The quantities taken are open to the workers' option and should be stated with the results.

Observation of the details that have been mentioned as to the preparation and storage of solutions along with a little experience and care will result in very satisfactory determinations. The determination of hydrogen-ion concentration has afforded the explanation and elimination of many troublesome and undesirable reactions in pharmaceutical products. There is at present a rapidly growing list of pharmaceuticals that are being held to a very definite acidity or alkalinity, and there can be no doubt that they deserve a preference.

ELI LILLY AND COMPANY, INDIANAPOLIS, INDIANA.

CARE OF ANIMALS FOR BIOLOGIC ASSAYS.

(*Concluded from p. 442, May* JOUR. A. PH. A.)

BY PAUL S. PITTENGER.

THE ALBINO RAT.

Feeding.—The albino rat thrives on almost any food utilized for human consumption. The more varied the diet the more vigorous the rats. The common