Brit. J. Pharmacol. (1947), **2**, 259.

THE INFLUENCE OF CALCIUM AND POTASSIUM IONS ON THE TOXICITY OF OUABAIN

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(Received April 22, 1947)

In the mass of literature dealing with digitalis and Ca and K ions there are no quantitative results showing the influence of varying amounts of these ions on the action of a constant dose of glycoside ; nor are there any laboratory observations on the effects of Ca or K on the symptom of digitalis vomiting. Accordingly the following studies have been made.

Anaesthetized rabbits have been used to determine (i) the lethal dose of ouabain infused intravenously at constant rate in physiological saline, (ii) the lethal dose of the same concentration of ouabain infused together with increasing concentrations of $CaCl_2$, and (iii) the lethal dose of the same concentration of ouabain infused together with increasing concentrations of KCl.

Perfused rabbit hearts have been used to determine the effect of alterations in the amount of (a) $CaCl_2$ and (b) KCl in the Locke solution on the effect of a fixed concentration of ouabain.

Pigeons have been used to determine whether the injection of KCl modified the action of ouabain in causing vomiting.

Experimental Results

(*a*) *Anaesthetized rabbits*

Method.—Thirty-four animals were used. All were given urethane by ear vein till full surgical anaesthesia was reached, the dose necessary being remarkably constant at 1.55 g. (6.2 ml. of 25 per cent (w/v) in distilled water) per kg. of rabbit given over 10–15 min. Urethane was used in order to avoid ether, which increases the scatter of results of digitalis assay by the cat method (Burn, 1937) ; the quantity agrees well with the 1.8 g./kg. usually recommended for cats intramuscularly. Cannulae were inserted into the trachea, the left carotid artery for recording the blood pressure by mercury manometer, and the left femoral vein for the infusion of solutions. Artificial respiration was given by a pump as soon as an animal's respiration began to be shallow. Into all animals, except controls, ouabain (20 μg./ml.) was infused in physiological saline at the constant rate of 0.15 ml./min./kg. rabbit. This rate of infusion is within the optimum range (Rapson and Underhill, 1935). Observations on the effects of Ca and K on the lethal dose of ouabain were made by adding varying amounts of $CaCl_2$ or KCl to the infusion, and the end-point of an infusion was taken as the point at which blood pressure suddenly fell to zero without recovery. The animals were thus in the following groups:

(i) Receiving ouabain alone.

(ii) Receiving ouabain + 1, 2 or 3 per cent (w/v) $CaCl_2$.

(iii) Receiving ouabain + 0.5, 1 or 2 per cent (w/v) KCl.
(iv) Controls receiving 3 per cent $CaCl_2$ alone.

The control animals needed more than 0.5 g. $CaCl_2$/kg. to kill them, in agreement with Nahum and Hoff (1937) who gave 10 per cent $CaCl_2$ at 2 ml./min. In the present series none of the rabbits receiving ouabain and calcium received more than 0.09 g. $CaCl_2$/kg. Since the effects of KCl on digitalis toxicity were protective, no controls with KCl alone were considered necessary.

Results.—In order to compare the relative effects of $CaCl_2$ and KCl on the action of ouabain, concentrations were expressed as molarities, but percentage concentrations (w/v) were also recorded for convenience (Table I). The mean results are represented graphically in Fig. 1 in order to show the difference

TABLE I

RABBITS. Femoral vein infusions of 20 μg. ouabain/ml. at 0.15 ml./kg./min.

Fluid infused		Individual LD ouabain μg./kg. rabbit	Mean
Ouabain and $CaCl_2$ Amount of $CaCl_2$			
Molarity × 10	Per cent		
2.7	3	30.5, 36.5, 35.7, 58.0, 58.0	43.7
1.8	2	60.0, 51.3, 73.5, 49.6, 74.7	61.8
0.9	1	75.5, 105, 93.3, 65.8, 56.0	79.1
Ouabain alone		114, 77.7, 81.0, 117, 88.0	95.5
Ouabain and KCl Amount of KCl			
Molarity × 10	Per cent		
0.67	0.5	148, 105, 114, 123	123
1.3	1	121, 145, 132, 166, 141	141
2.7	2	171, 151, 156, 130	152

in slope of the curves relating mean LD ouabain (ordinates) to concentration of $CaCl_2$ or of KCl (abscissae) in the infusion. Clearly there is a qualitative difference in effect, increased $CaCl_2$ causing a linear decrease in LD ouabain, while increase of KCl causes an increase in LD ouabain ; there also appears to be a quantitative difference, because the curve for KCl is the steeper. With the two lowest concentrations of potassium used, unit change of concentration had a greater effect on the LD of ouabain than had unit change in concentration of $CaCl_2$; thus, 1, 2, and 3 per cent $CaCl_2$ decreased the LD ouabain by 17, 36, and 54 per cent respectively, whereas 0.5, 1, and 2 per cent KCl increased it by 29, 47, and 59 per cent. This suggests that the action of ouabain is influenced more by the absolute concentration of potassium in a perfusion fluid than by the potassium/calcium ratio, at least over a certain range. In order to

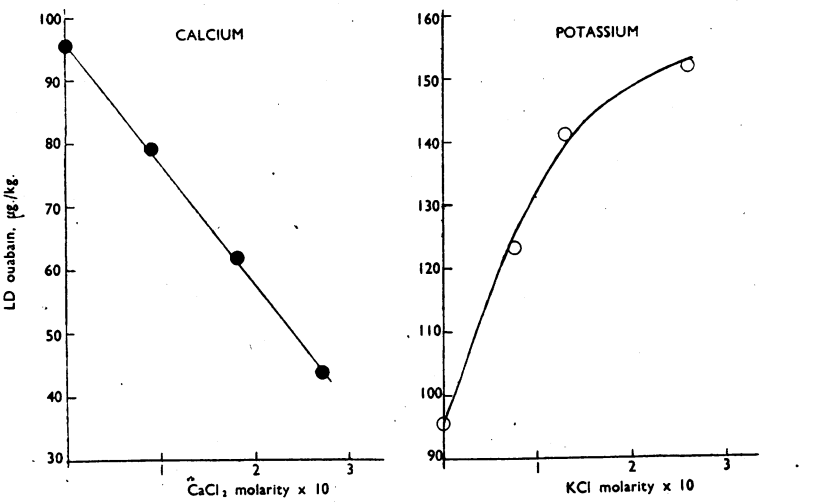

FIG. 1.—Rabbits. Femoral vein infusion of ouabain (20 µg./ml.) at a rate of 0.15 ml./kg./min. The relation between the LD ouabain and the Ca or K content of the infusion.

test this point it was necessary to perform experiments in which potassium or calcium concentrations could be lowered as well as raised, and accordingly the isolated perfused rabbit heart was used.

(*b*) *Perfused rabbit hearts*

Method.—Thirty-six animals were used. Animals were killed by a blow on the head and bled out by cutting the throat. The heart was rapidly cut out, dissected clean and perfused with oxygenated Locke's solution (percentage composition (w/v) as follows: 0.9 NaCl, 0.042 KCl, 0.024 $CaCl_2$, 0.1 dextrose, 0.05 $NaHCO_3$) at 36° by the Martin-Langendorff method. A hook was passed through the apex of the ventricles and the amplitude of beat recorded on a smoked drum by a lever and writing point. Rate and amplitude of beat and coronary flow were allowed to become steady, and from 30–45 min. after setting up the preparation perfusion was commenced with ouabain (0.4 µg./ml.) in the Locke's solution. The measure of toxicity of the ouabain was taken as the time it took to reduce the amplitude of beat to 50 per cent of its original value. This time is referred to as the "survival time." Thus anything which increased the toxicity of the ouabain *shortened* the survival time, and anything which decreased the toxicity *lengthened* the survival time. All hearts except the controls received 0.4 µg. ouabain/ml., and were divided into the following groups:

(i) Receiving ouabain in normal Locke's solution.

(ii) Receiving ouabain in Locke with half or twice the normal $CaCl_2$.

(iii) Receiving ouabain in Locke with 0.5, 1.25, and 1.5 times the normal KCl.

(iv) Control hearts receiving no ouabain. With the highest and lowest concentrations of $CaCl_2$ or KCl used all hearts survived for long periods.

(v) Hearts, in normal Locke containing 0.4 µg. ouabain/ml., in which toxic effects were caused to disappear by changing to perfusion fluid containing excess KCl.

Results.—For the reasons already given, the concentrations of $CaCl_2$ and KCl were expressed in molarities. These results (Table II) are plotted graphically (Fig. 2) as mean survival times against molar concentrations of $CaCl_2$ or KCl, and, as for the anaesthetized rabbits, it will be seen that when potassium is increased above normal, unit change in concentration of potassium produces a bigger effect on survival time than unit change in calcium concentration when calcium is decreased. This is expressed more clearly (Fig. 3), when the same

TABLE II

RABBIT HEARTS perfused by Langendorff's method with 0.4 μg. ouabain/ml.

Concentration in Locke $CaCl_2$		Concentration in Locke KCl		Ratio	Individual survival times in minutes	Mean survival times in minutes
Molarity × 10^3	mg. per 100 c.c.	Molarity × 10^3	mg. per 100 c.c.	K/Ca		
2.16	24	5.63	42	2.61	32, 39, 49, 50, 51, 34	38
4.32	48	,,	,,	1.30	23, 28, 14, 40, 13	24
1.08	12	,,	,,	5.22	60, 39, 45, 28, 49	44
2.16	24	2.81	21	1.30	20, 13, 14, 16	16
,,	,,	7.03	52.5	3.26	92, 110, 45, 48, 66	72
,,	,,	8.44	63	3.91	87, 114, 195, 207, 254	171

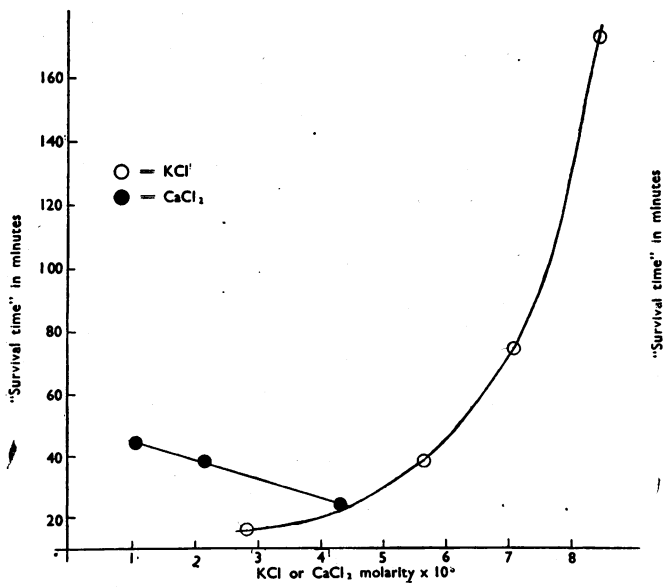

FIG. 2.—Langendorff rabbit hearts; 0.4 μg. ouabain/ml. Locke's solution at 36° C. The relation between the time required to reduce the amplitude of the beat to 50 per cent of its original value ("survival time") and the Ca or K content.

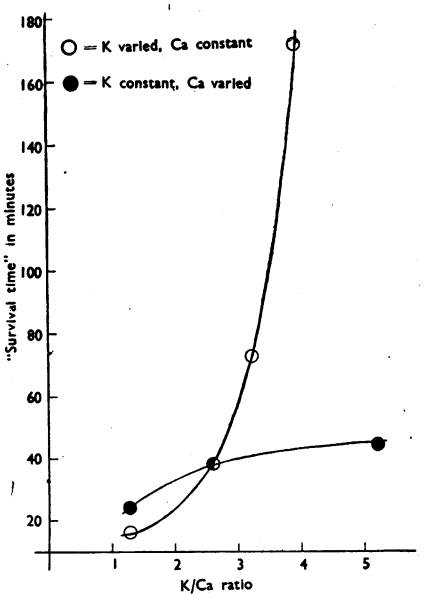

FIG. 3.—Same as Fig. 2. The relation between "survival time" and the K/Ca ratio.

survival times are plotted against the K/Ca ratio in the perfusion fluid. If the alteration in the ouabain effect had been solely a function of the K/Ca ratio, the same effect would have been obtained at a given K/Ca ratio whether it was attained by increasing K, or by decreasing Ca, but as Fig. 3 shows the absolute concentration of K had a relatively greater importance.

In addition to the quantitative results with potassium, three hearts were perfused with 0.4 μg. ouabain/ml. in normal Locke until gross irregularities of beat occurred, usually within 25 min.; potassium was then added to the perfusion fluid to increase its KCl content by 50 per cent. This abolished the irregular rhythms, caused systole to lessen, and prolonged the survival time, which would have been an hour at most, to several hours. A record of one of these hearts is shown in Fig. 4, and is in agreement with the results of Sampson *et al.* (1943) on the human being.

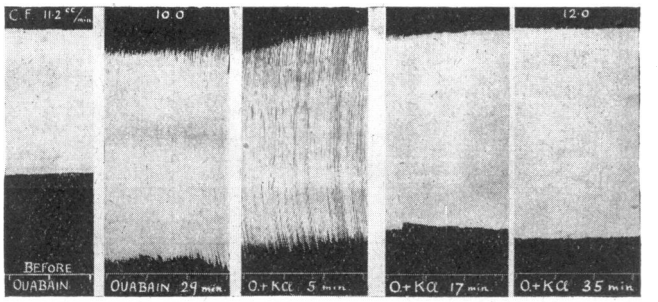

FIG. 4.—Langendorff rabbit heart. KCl abolition of irregular rhythm due to 0.4 μg. ouabain/ml. Tracing reads from left to right. Time marker in minutes. Between (i) and (ii) ouabain added to the Locke to contain 0.4 μg./ml. Between (ii) and (iii) KCl concentration raised by 50 per cent, with abolition of irregular beats (iv) and (v).

(*c*) *Pigeon emesis*

Apparently the effect of K on the vomiting produced by digitalis bodies has never been investigated, except for the observation of Sampson *et al.* (1943) that the nausea and vomiting of their patients who received an overdose of digitalis was not affected by potassium acetate administered orally. The pigeon-emesis method of digitalis assay introduced by Hanzlik and Schoemacher (1926), when modified as Burn (1930) suggested, affords a convenient method of testing this action of K against digitalis; if K were to lessen digitalis vomiting, this clearly would indicate antagonism of an extra-cardiac effect of digitalis, since digitalis vomiting has been shown to be independent of cardiac connections with the C.N.S. (Hanzlik and Wood, 1929; Haney and Lindgren, 1942).

Methods.—Twenty pigeons were injected with 15 μg. ouabain/300 g. pigeon into the wing vein on two separate occasions. The same pigeons were injected on two other occasions (interpolated between these two) with 15 μg. ouabain and 6 mg. KCl per 300 g. pigeon. This amount of KCl was found to be about the maximum which pigeons would tolerate, for in a trial with KCl alone it killed one bird in a group of 20. The number vomiting is recorded in Table III. When the pigeons were injected with ouabain alone 22 out of 40 injections caused vomiting; with ouabain and KCl, 19 out of 40 injections caused vomiting. Clearly potassium did not protect pigeons against the vomiting induced by the ouabain.

TABLE III

PIGEON EMESIS RECORD

Pigeon number	Ouabain 15 μg./300 g.		Ouabain 15 μg. and KCl 6 mg./300 g.	
	24.3.47	2.4.47	27.3.47	31.3.47
1	+	+	+	+
2	+	+	+	+
3	0	+	0	0
4	0	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	0	0	+
8	+	+	0	+
9	0	0	0	0
10	+	+	0	+
11	+	+	0	+
12	0	0	+	0
13	0	0	0	0
14	0	0	0	+
15	0	0	0	0
16	+	+	+	0
17	0	0	0	+
18	0	+	0	+
19	+	0	0	0
20	0	+	0	0
Total vomiting ..	10	12	7	12

DISCUSSION

Although there was reliable information concerning the effects of cardiac glycosides on frog hearts by the middle of the nineteenth century (Vulpian, 1855), it was not until after Ringer's proof of the importance of certain ions in the perfusion fluid (Ringer, 1883) that calcium and potassium became suspected of playing a part in the action of these glycosides. In frog hearts either Ca excess or K deficiency increased the action of digitalis, while Ca deficiency or K excess lessened its effect (Werschinin, 1910; Clark, 1912; Konschegg, 1913). From these results it was concluded that digitalis antagonized K just as Ca did. Some extreme views emerged; while Burridge (1915–16) postulated that the cardiac glycosides act by sensitizing the heart to calcium, Weizsäcker (1917) drew a similar conclusion that digitalis improves the force of the heart only when there is a lack of calcium. Loewi (1918) reiterated Burridge's contention, and others wrote in support (Geiger and Jarisch, 1922; Grumach; Grünwald; Handovsky; Hoffmann; Schoen; 1923). Pietrkowski (1918), however, maintained that the effects of low Ca on the action of digitalis could be countered by increasing the sugar of the perfusion fluid, and concluded that digitalis has a direct action on the heart. All these observers were concerned with the systolic action of digitalis, and with the systolic effect of calcium, whereas it has been shown (Werschinin, 1910; Cushny, 1925) that small doses of digitalis result in

diastolic arrest. Recently this observation has also been brought into line with the theory that digitalis sensitizes the heart to calcium (Blumenfeld and Loewi, 1945).

In mammals the occurrence of calcium and digitalis synergism has been well established both in animal experiments (Lieberman, 1932; Schunterman, 1935; Gold and Kwit, 1937; Gold and Edwards, 1927; Bower and Mengle, 1936; Golden and Brams, 1938), and in the human being, Edens and Huber (1916) finding that patients prone to digitalis pulse bigeminy have a high blood calcium, while Bower and Mengle (1936) record two cases of sudden death after intravenous calcium salts following digitalis.

In contrast to the foregoing results are those of Fischer (1928) who found that digitalis sensitizes the heart to all stimuli, e.g., Ca, ethyl alcohol, or K, and of Camp (1939) who also found the heart after treatment with digitalis to be sensitized to K. Nahum and Hoff (1937), however, and Smith, Winkler, and Hoff (1939) failed to obtain Ca and digitalis synergism. Nyiri and DuBois (1930) disagree with the extreme view of Loewi, maintaining that digitalis can exert its full effect in the complete absence of calcium in the fluid perfusing a frog's heart, though they agree that excess of calcium enhances its action.

A new line of evidence that K is involved in digitalis action arises first from the work of Calhoun and Harrison (1931). They showed that toxic doses of digitalis lower the level of cardiac K; the effect of therapeutic doses was doubtful. Any theory based on these results which suggests that digitalis acts by lowering the K/Ca ratio appears to be untenable, since Calhoun and Harrison also found, in fatal human cases with cardiac failure, that the myocardium of the dilated chambers was low in K. Confirmation of this effect of toxic doses has been obtained (Wood and Moe, 1938; Hagen, 1939; Wedd, 1939; Boyer and Poindexter, 1940) though these authors find the effect of therapeutic doses on K content is either negligible or else is to increase it. K loss by digitalis action is also recorded from frog skeletal muscle (Cattell and Goodell, 1937), while further evidence of an effect of digitalis on K metabolism in general is given by Zwemer and Lowenstein (1940), who found that digitalis lowers the plasma K and prolongs life in adrenalectomized animals, thus calling attention to the chemical similarity between the digitalis bodies, especially digitoxigenin, and cortin. Dorfman (1940), however, using adrenalectomized mice, was unable to demonstrate any cortin-like activity of strophanthin.

Therapeutic advantage has been taken of the antagonism between potassium and digitalis (Sampson, Albertson and Kondo, 1943) in order to alleviate the cardiac effects of overdosage of digitalis by giving oral doses of potassium acetate. No relief of nausea and vomiting was obtained, but visual disturbances disappeared.

The most recent publications concerning digitalis and heart biochemistry (Chen and Geiling, 1947) show that toxic doses of digitalis diminish cardiac

adenosine triphosphate (ATP), phosphocreatine and adenylic acid, while therapeutic doses have no such effect, and in decompensated hearts the re-synthesis of these three substances was hastened by digitalis administration (Weicker, 1935). These facts, together with the observation that the isolated perfused rabbit heart in systolic contracture from digitalis can be temporarily restored by ATP (Chen and Geiling, 1946), tempt speculation that the K/Ca effects of the cardiac glycosides may exert an influence on the intricacies of the higher energy-liberating phases of the chemical reactions concerned with muscle contraction.

The experiments now described give results which agree with those of the majority; increased calcium potentiates, and increased potassium antagonizes digitalis glycosides. The quantitative aspect takes the matter further, demonstrating the relative importance of the absolute concentration of potassium in the cardiac action of the glycosides, while the failure to protect pigeons against their emetic effect suggests that different biochemical mechanisms are concerned in the cardiac and emetic actions at least.

The ability of potassium salts not only to delay the toxic action of digitalis, but also to remove toxic effects when already developed is not generally known. Sampson and his colleagues (1943) gave 5–10 g. potassium acetate by mouth as a 25 per cent solution to a series of 14 patients in whom digitalis had produced ectopic beats which were recorded by the electrocardiograph. Only one dose was given on any one day. The authors followed the rise in serum potassium and observed the disappearance of the ectopic beats. This occurred in every patient and outlasted the change in serum potassium. The observations described in this paper add support to these findings and suggest that they are due to the interplay of potassium and digitalis in the heart muscle itself.

Summary

1. The LD of ouabain by intravenous infusion in physiological saline was determined on 34 rabbits under urethane.

2. The effect of $CaCl_2$ and of KCl on the LD of ouabain was observed by adding them to the perfusion fluid: 1, 2, and 3 per cent solutions of $CaCl_2$ decreased the LD of ouabain by 17, 36, and 54 per cent respectively; 0.5, 1, and 2 per cent solutions of KCl increased the LD by 29, 47, and 59 per cent respectively.

3. Survival times were observed of 36 Langendorff rabbit hearts perfused at 36° C. with Locke's solution containing 0.4 μg. ouabain/ml. The calcium or potassium concentration was varied in different experiments.

4. Increased calcium or decreased potassium shortened survival time, while decreased calcium or increased K lengthened it.

5. The effects of altering the potassium were greater than those of corresponding changes in calcium. Halving the $CaCl_2$ prolonged mean survival time by 6 min.; increasing the KCl by 50 per cent prolonged it by 133 min.

6. In a group of 20 pigeons injected via the wing vein with ouabain (15 μg./300 g. pigeon) on two occasions, 22 out of 40 injections caused vomiting. On two other occasions with the same dose of ouabain plus KCl (6 mg./300 g. pigeon), 19 out of 40 injections caused vomiting. The difference is not significant.

I wish to thank Prof. Burn for the guidance and advice he has given throughout.

REFERENCES

Blumenfeld, S., and Loewi, O. (1945). *J. Pharmacol.*, **83**, 96.
Bower, J. O., and Mengle, H. A. K. (1936). *J. Amer. med. Ass.*, **106**, 1151.
Boyer, P. K., and Poindexter, C. A. (1940). *Amer. Heart J.*, **20**, 587.
Burn, J. H. (1930). *J. Pharmacol.*, **39**, 221.
Burn, J. H. (1937). *Biological Standardization*, p. 232. Oxford University Press.
Burridge, W. (1915–16). *Quart. J. Med.*, **9**, 271.
Calhoun, A. J., and Harrison, T. R. (1931). *J. clin. Invest.*, **10**, 139.
Camp, W. J. R. (1939). *Arch. intern. Pharmacodyn.*, **61**, 60.
Cattell, M., and Goodell, H. (1937). *Science*, **86**, 106.
Chen, G., and Geiling, E. M. K. (1946). *Fed. Proc.*, **5**, 170.
Chen, G., and Geiling, E. M. K. (1947). *Schweiz. med. Wschr.*, **77**, 25.
Clark, A. J. (1912). *Proc. roy. Soc. Med.*, **5**, III, 181.
Cushny, A. R. (1925. *Digitalis and its Allies*, p. 40. London: Longmans Green and Co.
Dorfman, R. I. (1940). *Proc. Soc. exp. Biol.*, **43**, 489.
Edens, E., and Huber, J. E. (1916). *Dtsch. Arch. klin. Med.*, **118**, 476.
Fischer, H. (1928). *Arch. exp. Path. Pharmakol.*, **130**, 194.
Geiger, E., and Jarisch, A. (1922). *Arch. exp. Path. Pharmakol.*, **94**, 52.
Gold, H., and Edwards, D. (1927). *Amer. Heart J.*, **3**, 45.
Gold, H., and Kwit, N. (1937). *Science*, **86**, 330.
Golden, J. S., and Brams, W. A. (1938). *Ann. intern. Med.*, **11**, 1084.
Grumach, H. (1923). *Arch. exp. Path. Pharmakol.*, **98**, 123.
Grünwald, H. F. (1923). *Arch. exp. Path. Pharmakol.*, **97**, 156.
Hagen, P. S. (1939). *J. Pharmacol.*, **67**, 50.
Handovsky, H. (1923). *Arch. exp. Path. Pharmakol.*, **97**, 171.
Haney, H. F., and Lindgren, A. J. (1942). *J. Pharmacol.*, **76**, 363.
Hanzlik, P. J., and Schoemacher, H. A. (1926). *Proc. Soc. exp. Biol.*, **23**, 298.
Hanzlik, P. J., and Wood, D. A. (1929). *J. Pharmacol.*, **37**, 67.
Hoffmann, H. (1923). *Arch. exp. Path. Pharmakol.*, **96**, 105.
Konschegg, A. von (1913). *Arch. exp. Path. Pharmakol.*, **71**, 251.
Lieberman, A. L. (1932). *J. Pharmacol.*, **47**, 183.
Loewi, O. (1918). *Arch. exp. Path. Pharmakol.*, **82**, 131.
Nahum, L. H., and Hoff, H. E. (1937). *Proc. Soc. exp. Biol.*, **36**, 380.
Nyiri, W., and DuBois, L. (1930). *J. Pharmacol.*, **39**, 111.
Pietrkowski, G. (1918). *Pflüg. Arch. ges. Physiol.*, **172**, 497.
Rapson, G. N., and Underhill, S. W. F. (1935). *Quart. J. Pharm. Pharmacol.*, **8**, 409.
Ringer, S. (1883). *J. Physiol.*, **4**, 29.
Sampson, J. J., Albertson, E. C., and Kondo, B. (1943). *Amer. Heart J.*, **26**, 164.
Schoen, R. (1923). *Arch. exp. Path. Pharmakol.*, **96**, 158.
Smith, P. K., Winkler, A. W., and Hoff, H. E. (1939). *Arch. intern. Med.*, **64**, 322.
Schunterman, C. E. (1935). *Z. ges. exper. Med.*, **96**, 520.
Vulpian (1855). *Gaz. med. Paris*, 559.
Wedd, A. M. (1939). *J. Pharmacol.*, **65**, 268.
Weicker, B. (1935). *Arch. exp. Path. Pharmakol.*, **178**, 524.
Weizsäcker, V. von (1917). *Arch. exp. Path. Pharmakol.*, **81**, 247.
Werschinin, N. (1910). *Arch. exp. Path. Pharmakol.*, **63**, 386.
Wood, E. H., and Moe, G. K. (1938). *Amer. J. Physiol.*, **123**, 219.
Zwemer, R. L., and Lowenstein, B. E. (1940). *Science*, **91**, 75.