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ACTIONS OF SODIUM AZIDE

BY

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Hydrazoic acid or azoimide is a colourless liquid with explosive properties which solidifies at −80° C. and boils at 37° C. Azoimide gas has a characteristic pungent odour. First prepared by Curtius (1890) it is a triazo compound in which a complex of three nitrogen atoms acts as a monad radical. Hendricks and Pauling (1925) showed that the N_3 ion is constituted $N \rightleftharpoons N \rightleftharpoons N$ in chain form. Hydrazoic acid is a feebly dissociating acid. Smith and Wolf (1904) showed that it was toxic on inhalation by small animals, causing a fall in blood pressure, tachycardia, and stimulation of respiration, and that in solution it paralysed preparations of isolated frog muscle. Biehler (1927) observed that the gas was a convulsant in frogs, and Hildebrandt and Schmidt (1937) extended the observation to cats and also noted a stimulant effect on gut. Stern (1927) and Kocher (1930) described acute collapse in human beings from inhalation of the gas, and Graham, Robertson, and Rogan (1948) studied symptoms of hypotension in a group of workmen exposed to the fumes of azoimide intermittently over a period of years. Hydrazoic acid forms explosive salts with heavy metals, the lead salt being used as a detonator. Fairhall, Jenrette, Jones, and Pritchard (1943) have discussed the hazards of this substance as an industrial poison.

The azoimide salt of sodium is stable, neutral, and fully dissociated in solution (West, 1900). Loew (1891) published an account of experiments on the toxicity of sodium azide, in which he showed that approximately 40 mg./kg. was lethal to mice, while 30 mg. killed a dog in 104 min. from respiratory failure and cramp. Smith and Wolf (1904) showed that sodium azide had the same effect as azoimide in lowering blood pressure, stimulating respiration, and increasing the rate of the heart. They found that the isolated perfused heart of the rabbit was inhibited by a concentration of 1 in 90,000. Further observations have been reported by Graham (1948).

Most recent work with the azide radical has been confined to the investigation of the effect of sodium azide on cellular respiration. Keilin (1936), Keilin and Hartree (1934 and 1935), and Stannard (1939) have shown that azide interferes with cellular metabolism by inhibiting the oxidation processes in which cytochrome plays a part; it also inhibits indophenol oxidase and liver catalase, but not glycolysis. The salt is fungistatic and forms a compound with methaemoglobin *in vitro* but not *in vivo*.

Toxicity

The LD50 in groups of white mice was found to be 28–34 mg./kg. by intraperitoneal injection, 19 mg./kg. intravenously, and 27 mg./kg. by mouth. The LD5 i.p. was 23.7 mg./kg. The LD50 of sodium nitrite was 168 mg./kg. and of potassium thiocyanate 600 mg./kg. measured at the same time. According to Fairhall *et al.* (1943) the LD75 of NaN_3 for rats is 75 mg./kg. i.p.

Mice, rats, guinea-pigs, and rabbits injected with sodium azide by the oral, subcutaneous, intramuscular, intraperitoneal, or intravenous routes all showed similar symptoms, which varied only in degree and rapidity of onset with varying dosage levels of the salt. Sublethal doses caused a preliminary stimulation of respiration and might give rise to clonic convulsions. This phase was accompanied by increased urination and passage of faeces and was followed by a prolonged period of collapse such as is seen with nitrite and thiocyanate. Death occurred quietly with azide if the dose was such as to delay the termination for some hours, but if the dose was overwhelming death occurred acutely in convulsions. In these circumstances mice and rats assumed a character-

istic death posture with the head flexed, the forelimbs flexed and pronated, and the tail and hind limbs extended.

Cardiovascular System

Systemic blood pressure.—Small doses of sodium azide caused a sharp transient fall in arterial blood pressure in cats and rabbits, the former being more sensitive than the latter. The amount of this fall depended on the initial level of the blood pressure, the anaesthetic used, and the dose of azide given. Cats were most sensitive when anaesthetized with ether and given aqueous hydrazoic acid intravenously: under these conditions a fall of blood pressure of about 60 mm. Hg was observed with 0.5–1.0 μg. HN_3 per kg. In rabbits under urethane 5–100 μg. HN_3/kg. was needed to produce a similar effect.

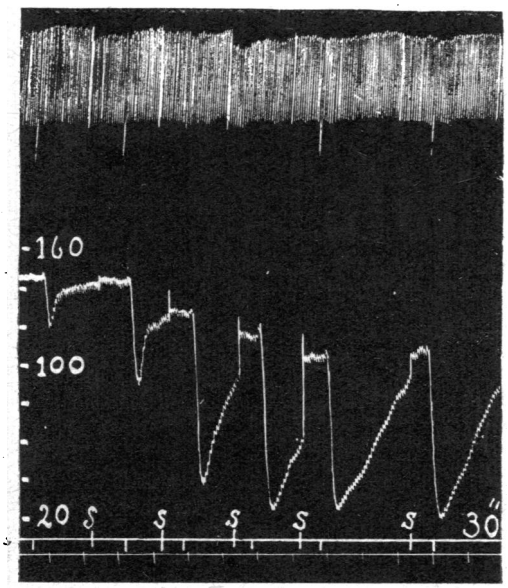

FIG. 1.—Cat ♂ 3.4 kg. Chloralose, 80 mg./kg. i.v. Upper line respiration (stethograph lever), next line carotid blood pressure, injection signal and drum stop (S), base line for zero blood pressure and time in 30 sec. Transient fall in blood pressure and stimulation of respiration occurs on each injection of 2 μg. NaN_3 at intervals of 5 min. The effect on respiration is minimal: the effect on blood pressure increases on repetition.

If the dose of sodium azide given intravenously to cats was small (1.0 μg./kg. or less) the response increased with repetition until it became constant, and the pressure did not return to its former level between doses (see Fig. 1). If the initial dose was larger (or in rabbits) this early increase in response with repetition of doses was not seen.

There was no diminution of response to repeated injections of sodium azide (5–15 μg./kg.) so long as the blood pressure was permitted to return to its initial level between doses. If the time interval between administrations of the azide was shortened or the dose given at the same intervals of time was increased, the blood pressure did not return to its original level. In such circumstances the effects of repeated doses of azide decreased and might be extinguished. As soon as time was given for restoration of the pressure approximately to its initial level the full effect of the original dose of azide was restored. This relationship between the initial level of the blood pressure and the response is illustrated in Fig. 2.

The fall in blood pressure resulting from administration of small doses of azide to cats or rabbits probably results from a direct action on the smooth muscle of the blood vessels. It occurred in anaesthetized eviscerated animals after atropine and vagotomy, after benadryl, and after excision of the carotid sinuses and adrenalectomy, and in spinal cats similarly prepared. The effect was not modified by previous injection of eserine or nicotine. Records of the volume of the spleen and foreleg showed a decrease in the volume of both accompanying the fall in pressure, but in three preparations out of ten the spleen dilated actively. Perfusion of the hind limbs of the rat through the aorta with a saline solution

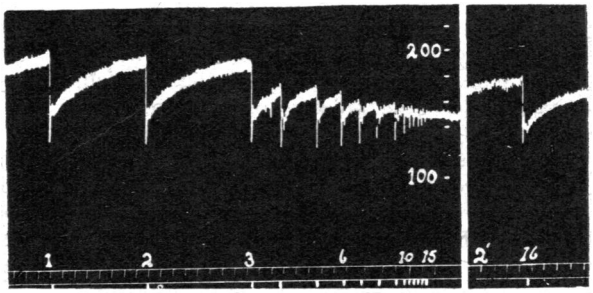

FIG. 2.—Cat ♂ 3.2 kg. Chloralose, 80 mg./kg. i.v. Upper line carotid blood pressure, middle line time in 2 min., lower line zero blood pressure and injection signal. 50 μg. NaN_3 in 0.25 ml. water was injected quickly at each signal. Nos. 1–3 were given at 15 min. intervals, nos. 4–6 at 5 min. intervals, nos. 7–10 at 2 min. intervals, nos. 11–15 at lesser intervals. Between nos. 15 and 16 an interval of 45 min. The degree of response to azide is related to the initial level of blood pressure.

under constant pressure and recording of the volume of the venous effluent showed that azide increased the rate of flow as a result of vasodilatation.

If the dose of sodium azide injected into cats was increased to 10–20 mg./kg. a rise in blood pressure occurred in the majority of animals, though some reacted with a severe and prolonged fall in pressure. The rise in blood pressure usually obtained was not modified by excision of the carotid sinus area or vagotomy. It was abolished by previous excision of the adrenal glands or by blocking agents such as dibenamine and dihydroergotamine. That the stimulation of the adrenals is an indirect one was proved by failure to repeat the effect after bilateral splanchnic nerve section and by the failure to obtain

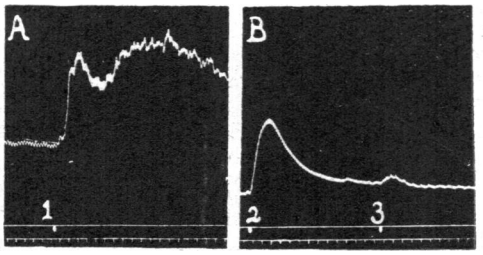

FIG. 3.—Cat ♂ 3.2 kg. Ether, chloralose 80 mg./kg. Upper line carotid blood pressure, middle line zero blood pressure and injections, lower line time in 10 sec. Preceding the records shown the cat received 10 mg. NaN_3/kg. i.v. (with effect as in A), and bilateral excision of the carotid sinuses. At 1 repeat of 10 mg. NaN_3/kg. giving prolonged rise in blood pressure. Between A and B bilateral splanchnic nerve section. At 2 adrenaline (2 μg./kg.). At 3 repeat 10 mg. NaN_3/kg. with no effect.

the effect in spinal cats (see Fig. 3). A similar rise in pressure was observed in rabbits after similar doses of azide.

The heart.—Injection of 50 μg.–1.0 mg. sodium azide into the inflow of the perfused heart of the cat (Langendorff preparation) caused an increase in the force but not in the rate of contraction: 2–5 mg. caused transient inhibition of some preparations. In the isolated rabbit heart the increase in force was less marked and inhibition was more often seen with the higher doses. In both species dilatation of the coronary vessels with increase in coronary flow occurred. Fig. 4 shows the effect of a dose of 50 μg. sodium azide on the perfused cat heart and the coronary flow from it. In view of the hypotensive effect of sodium azide on systemic blood pressure the action of other agents with a similar hypotensive activity was tested on the coronary circulation. The dilator effect of an equimolar amount of sodium nitrite was less intense but longer in duration than that of 50 μg. sodium azide; potassium thiocyanate had no effect (see Fig. 5).

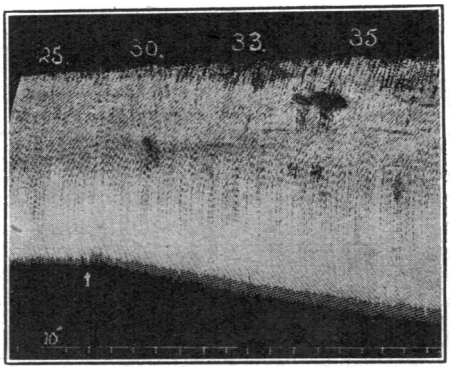

FIG. 4.—Record of the action of a perfused cat heart (Langendorff preparation). Time in 10 sec. The figures at the top are a measure of the coronary flow in ml./min. At the arrow 50 μg. NaN_3 was injected into the perfusing cannula.

The effect of intravenous injection of sodium azide on the heart of anaesthetized cats and rabbits was variable. In rabbits anaesthetized with urethane 10–100 μg. of azide increased the force and sometimes the rate of contraction of the heart, but in the majority of animals the heart was temporarily slowed. Atropine or vagotomy abolished this slowing but left the increase in force

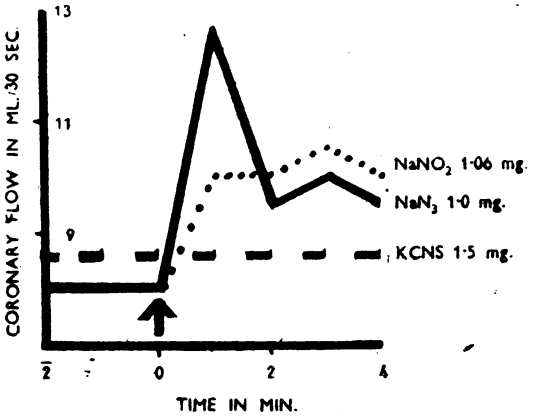

FIG. 5.—Graph of the coronary flow in the isolated perfused heart of the cat (Langendorff preparation). At intervals of 20 min. equimolar amounts of potassium thiocyanate, sodium nitrite, and sodium azide were injected (arrow). KCNS had no effect (interrupted line), NaN_3 had a powerful but short-lasting dilator effect (solid line), and $NaNO_2$ had a longer-lasting effect (dotted line).

unaffected. Larger doses of azide (5–10 mg. of azide/kg.) produced a marked initial slowing and inhibition of the heart followed by increase in cardiac activity above the initial level. This slowing was abolished by vagotomy or atropine, leaving the stimulation unaffected. In cats the effects of azide were similar, but this animal was more sensitive to sodium azide than the rabbit. A few μg. of azide per kg. brought about a marked increase in the rate and force of the heart (see Fig. 6): larger doses (5 mg./kg.) caused a transient inhibition, abolished by atropine or vagotomy and unobtainable in the spinal cat, followed by a prolonged increase in the rate and force of the heart.

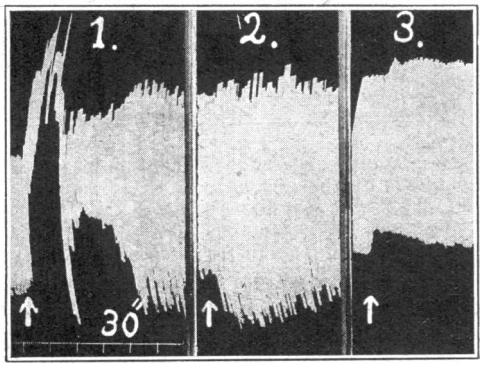

FIG. 6.—Direct myocardiograph tracing from cat ♂ 3.2 kg., ether and 80 mg. chloralose/kg. 1. Sodium azide (5 mg./kg.): transient inhibition followed by stimulation. 2. Repeat after bilateral cervical vagotomy and 1 mg. atropine sulphate/kg., followed by 15 mg. dibenamine HCl/kg.: stimulation without preliminary inhibition. 3. Repeat after bilateral adrenalectomy: absence of stimulation.

not affected by dibenamine (15 mg./kg.) but prevented by previous adrenalectomy (see Fig. 6). In a certain number of these animals, as already mentioned in the section dealing with the effects of azide on the blood pressure, the effect of large doses of the salt were mostly inhibitory with a fall in pressure replacing the rise in pressure. In these circumstances the cardiac action was also inhibited for a long period after injection, and adrenalectomy made little or no difference to the reactions obtained. Excision of the carotid sinus area did not modify these responses.

Since sodium azide in small doses appeared to have a more powerful effect than the other commonly used hypotensive agents on the blood flow in the systemic and coronary circulations comparison was made of their activities on the heart of the whole animal under light anaesthesia (ether, chloralose, or urethane). As is shown in Fig. 7 the

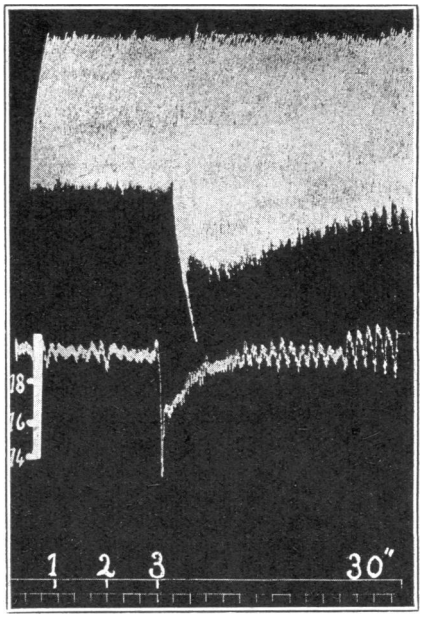

FIG. 7.—Cat ♂ 4 kg., 80 mg. chloralose/kg. i.v. Upper line direct myocardiograph lever, next line carotid blood pressure, injection signal, time in 30 sec. At 1, 2, and 3 injection of equimolar amounts of KCNS, $NaNO_2$, and NaN_3 (50 μg.) respectively. Note transient inhibition of heart followed by augmentation of the beat and abrupt fall in blood pressure after injection of azide.

characteristic cardiovascular action of azide—stimulation of the heart and fall in blood pressure—is obtained with amounts of azide such that equimolar amounts of nitrite and thiocyanate have no obvious effect.

Respiration

The rate and depth of breathing were increased by inhaled hydrazoic acid gas in very low concentrations (see Graham, Robertson, and Rogan, 1948, Fig. 1) and by injected azide in all doses. With doses of less than 1 μg. sodium azide per kg. the stimulant effect on respiration did not last so long as the accompanying fall in blood pressure. The degree of stimulation might be such as to cause marked irregularity of breathing and even generalized clonic convulsions. With suitable doses these convulsions appeared immediately after injection of the azide and were not asphyxial in origin, but doses of azide insufficient to give rise to a convulsive response might, after a variable period of stimulation and irregularity of breathing, bring about a depression and gradual failure of respiration; this was often complicated by terminal asphyxial convulsions.

Other actions

Sodium azide dilated the perfused bronchus of guinea-pigs and stimulated both isolated and intact intestine of rabbit, guinea-pig, and cat. In anaesthetized animals this stimulation of the gut, accompanied by flushing with arterial blood, was obvious: records of the movements of an intestinal loop and of the pressure within it revealed a transient relaxation of the muscle preceding the increase in activity. The isolated uterus of rat, rabbit, and guinea-pig was not affected by azide, but that of the cat was stimulated. In the pregnant cat under ether, however, this effect was negligible with amounts of azide which caused a prolonged fall in blood pressure: the urinary bladder contracted vigorously and expelled urine. There was no difference in the diuretic response of groups of rats injected with small doses of azide after receiving water by mouth and control animals not given azide, but if the dose of azide was increased the rats showed evidence of collapse and urine production ceased. Frequency of micturition as seen in intact animals given azide is not due to diuresis but to the action on the urinary bladder, probably a direct one. Azide is water soluble and was absorbed from all routes of administration. It did not discolour the blood with methaemoglobin as did nitrite: like nitrite it was not excreted unchanged in the urine, which failed to give the brown colour reaction with ferric chloride obtained *in vitro* with dilute sodium azide before injection or feeding.

Discussion

The actions of sodium azide on the cardiovascular system of mammals are similar to those of sodium nitrite. The effect of inhaled amyl nitrite on the systemic blood pressure as described by Bradford and Dean (1894) is similar to that of hydrazoic acid gas as described by Smith and Wolf (1904) and elaborated by Graham, Robertson, and Rogan (1948). That the tachycardia caused by nitrite is a reflex one following upon the fall in blood pressure was shown by Dossin (1911), whose findings with the action of nitrite on the isolated perfused heart are paralleled by those described above for azide. Loeb (1904) showed that nitrite is a coronary dilator, and Boyer, Wégria, and Green (1939) and Essex, Wégria, Herrick, and Mann (1940) confirmed this finding by modern techniques. The same action has been demonstrated with azide. Both nitrite and azide stimulate the respiration, but Heymans, Bouckaert, and Dautrebande (1931) attribute to the carotid sinus a larger part in the reflex stimulation of respiratory and cardiovascular mechanisms by nitrite than the present work indicates is played by the carotid sinus in the action of azide. Leech (1893) and Smith and Wolf (1904) have shown respectively that nitrite and azide inhibit the contractile power of isolated frog gastrocnemius muscle. According to Beams and Barlow (1932) nitrite causes a contraction and then a relaxation of isolated strips of rabbit gut in Locke's solution: azide causes a contraction *in vitro* and a relaxation followed by a contraction *in vivo*. Both substances dilate the perfused bronchi. Both are modified by metabolic processes in the body: their fate is as yet unknown.

The main differences lie in the greater direct effect of azide on the central nervous system, especially on the respiratory centre, the more powerful action of azide on the peripheral vascular bed, the absence of formation of methaemoglobin in the blood stream of animals receiving azide intravenously, and the proven effect of azide on various enzymatic processes in the living cell.

In human beings it has been shown that hydrazoic acid fumes lower systolic and diastolic blood pressure to a profound extent for some three to six hours with the production of a mild degree of headache.

The mechanism of action of azide is complex. Peripherally it relaxes the smooth muscle of blood vessels and bronchi while increasing the force of the cardiac contraction. This results in a greatly increased coronary flow accompanying a fall in systemic blood pressure. Centrally it stimulates the medulla, which results in a stimulation of respiration, an initial vagal inhibition of the heart, and a subsequent sympathetic stimulation of the heart. Azide also has a direct stimulant action on cardiac muscle which may play a part in this phenomenon. The resultant effect of these opposing forces is determined by the species and state of the animal, especially the initial blood pressure, the sensitivity of its nervous system and reflex mechanisms, the anaesthetic administered, and the dose and rate of administration of the azide. The larger doses of azide (mg. rather than μg. /kg.) powerfully affect the sympathetico-adrenal mechanism and cause a prolonged rise in systemic blood pressure and a great increase in cardiac force, probably brought about by a release of adrenaline from the suprarenal glands. The variable effects on the volume of the spleen and the leg are the result of these conflicting mechanisms. The activity of gut and bladder is increased, that of the uterus is little altered. Stimulation of the central nervous system, which may be severe

enough to cause a characteristic convulsive seizure, is in other cases followed by depression leading to asphyxia from respiratory failure.

SUMMARY

Sodium azide, a neutral stable salt of hydrazoic acid, is a potent hypotensive agent which dilates peripheral blood vessels by direct action. It stimulates cardiac muscle and dilates the coronary vessels directly.

It affects the rate and force of the mammalian heart *in vivo* by stimulating the vagal and sympathetic cardiovascular mechanisms: such effects are produced centrally rather than by carotid sinus reflexes.

It stimulates respiration and in large doses produces generalized convulsions followed by respiratory depression.

It increases gut and bladder contractions but hardly affects the uterus.

The general action is similar to that of sodium nitrite, but azide is more powerful.

The LD50 i.p. in white mice was 28–34 mg./kg. when that of sodium nitrite was 168 mg./kg.

It does not produce methaemoglobin *in vivo* and is excreted as a metabolite which no longer gives a brown colour reaction when ferric chloride is added to the urine.

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REFERENCES

Beams, A. J., and Barlow, O. W. (1932). *Arch. intern. Med.*, **49**, 276.
Biehler, W. (1927). *Arch. exp. Path. Pharmak.*, **126**, 1.
Boyer, N., Wégria, R., and Green, H. D. (1939). *Amer. J. Physiol.*, **126**, 440.
Bradford, J. R., and Dean, H. P. (1894). *J. Physiol.*, **16**, 34.
Curtius, T. (1890). *Ber. dtsch. chem. Ges.*, **23**, 3023.
Dossin, F. (1911). *Arch. int. Pharmacodyn.*, **21**, 425.
Essex, H. E., Wégria, R., Herrick, J. F., and Mann, F. C. (1940). *Amer. Heart J.*, **19**, 554.
Fairhall, L. T., Jenrette, W. V., Jones, S. W., and Pritchard, E. A. (1943). *Pub. Health Rep., U.S.*, **58**, 607.
Graham, J. D. P. (1948). *Arch. int. Pharmacodyn.*, **77**, 40.
Graham, J. D. P., Robertson, D. S., and Rogan, J. M. (1948). *Amer. J. Hyg.*, **30**, 98.
Hendricks, S. B., and Pauling, L. (1925). *J. Amer. chem. Soc.*, **47**, 2904.
Heymans, D., Bouckaert, J. J., and Dautrebande, L. (1931). *C.R. Soc. Biol., Paris*, **106**, 1279.
Hildebrandt, F., and Schmidt, K. F. (1937). *Arch. exp. Path Pharmak.*, **187**, 155.
Keilin, D. (1936). *Proc. Roy. Soc.*, **121B**, 165.
Keilin, D., and Hartree, E. F. (1934). *Nature*, **134**, 933.
Keilin, D., and Hartree, E. F. (1935). *Proc. Roy. Soc.*, **117B**, 1.
Kocher, S. (1930). *Berl. klin. Wschr.*, **2**, 2160.
Leech, D. J. (1893). *Brit. med. J.*, **1**, 1305.
Loeb, O. (1904). *Arch. exp. Path. Pharmak.*, **51**, 64.
Loew, O. (1891). *Ber. dtsch. chem. Ges.*, **24**, 2947.
Smith, L., and Wolf, C. G. L. (1904). *J. med. Res.*, **12**, 451.
Stannard, J. N. (1939). *Amer. J. Physiol.*, **129**, 195.
Stern, R. (1927). *Berl. klin. Wschr.*, **1**, 304.
West, R. (1900). *J. chem. Soc.*, **77**, 705.