THE PHYSIOLOGICAL ACTION OF SOME CAMPHENE PHOSPHONATES OF SODIUM

By J. A. GARDNER AND W. L. SYMES.

*From the Physiological Laboratory of the University of London,
South Kensington*

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I. INTRODUCTION

In a paper published in 1894,¹ Marsh and Gardner showed that when camphene is treated in the cold with phosphoric pentachloride, one atom of hydrogen of the camphene is replaced by the grouping PCl_4 with the formation of a compound of the formula $\text{C}_{10}\text{H}_{15}\text{PCl}_4$, which on treatment with water yielded two acids— α - and β -camphene-phosphonic acids. The α -camphene phosphonic acid crystallised from dilute alcohol in colourless fluffy needles, insoluble in ether, but soluble in alcohol, benzene and chloroform. The air-dried acid had the composition represented by the formula $2\text{C}_{10}\text{H}_{15}\text{PO}_3\text{H}_2 + \text{H}_2\text{O}$. When dried at 100° it lost $2\text{H}_2\text{O}$, leaving the anhydro-acid $(\text{C}_{10}\text{H}_{15}\text{PO}_2\text{H})_2\text{O}$. The acid was found to be monobasic, and yielded a sodium salt of the formula $\text{C}_{10}\text{H}_{15}\text{PO}_3\text{HNa} + 4\text{H}_2\text{O}$. When heated at 100° the salt loses its water of crystallisation, two molecules of the salt losing $9\text{H}_2\text{O}$ and leaving the salt of the anhydro-acid $(\text{C}_{10}\text{H}_{15}\text{PO}_2\text{Na})_2\text{O}$.

β -camphene-phosphonic acid has the formula $\text{C}_{10}\text{H}_{15}\text{PO}_3\text{H}_2$, and crystallises from dilute alcohol in large colourless needles. It is soluble in ether, but insoluble in chloroform and benzene. It melts with decomposition at 167° , and does not yield an anhydro-acid. Like the α -acid, it is monobasic and yields a sodium salt which crystallises from water in unctuous plates having the formula $\text{C}_{10}\text{H}_{15}\text{PO}_3\text{NaH} + 5\text{H}_2\text{O}$. At 100° this salt loses nearly the whole of its water of crystallisation, but the last traces are difficult to remove at a temperature below that at which the salt itself undergoes decomposition.

1. *Trans. Chem. Soc.*, London, p. 35, 1904.

The α - and β -acids have not been converted the one into the other.

The β -sodium camphene-phosphonate decomposes quantitatively at 160° C. into camphene and sodium metaphosphate. The α -salt is more stable; on heating it yields the salt of the anhydro-acid, which does not decompose below 260° C., and then the decomposition is of a more profound nature.

On hydrolysis with water in sealed tubes both salts decompose with the formation of camphene and sodium hydrogen phosphate. Both salts are decomposed by bromine water with the liberation of phosphoric acid and the formation of bromo derivatives of camphene. The phosphonic acids derived from different camphenes differ in their rotatory power in respect of polarised light, but do not differ materially in chemical properties.

The above-mentioned properties may be explained on either of two views: (1) The replacement of OH of orthophosphoric acid by a camphene residue gives rise to two isomeric acids of the formula $O=P\left\langle \begin{smallmatrix} C_{10}H_{15} \\ OH \end{smallmatrix} \right\rangle_2$ of which the α -acid readily loses water on heating, passing into the pyro-form, while the β - does not do this at any temperature below which it decomposes; or (2) the α -acid may be regarded as derived from pyrophosphoric acid by the replacement of two hydroxyls by camphene

residues thus $O=P\left\langle \begin{smallmatrix} C_{10}H_{15} \\ OH \end{smallmatrix} \right\rangle_2$, and the β - as derived from ortho-

phosphoric acid as above. If the latter explanation is correct, the formula of the acid and its sodium salt would be respectively $(C_{10}H_{15}PO_2H)_2O + 2H_2O$ and $(C_{10}H_{15}PO_2Na)_2O + 9H_2O$, and further, we should expect that the β -acid and its salts should be convertible into the α -acid and its salts, which has so far proved not to be the case.

If both acids are regarded as derived from orthophosphoric acid, the cause of the isomerism must be looked for in the different positions which the phosphoric grouping occupies in each case with respect to the camphene nucleus.

In view of the investigations of Gamgee and his pupils on the physiological action of the ortho-phosphate and pyro-phosphate of soda, it was thought possible that an investigation of the physiological action of the α - and β -sodium camphene-phosphonates might throw light on these points, and in any case would yield possibly interesting information as to the effect of the introduction of a camphene group in

the molecule, the new substance being as readily soluble as that from which it was derived.

The following observations deal with some of the physiological properties of these bodies:—

The chemical portion of the research is the work of Gardner, whilst the physiological side was, in the early part of 1909, commenced by the late Professor Gamgee. On Professor Gamgee's death, after a few preliminary observations, the physiological work was undertaken by Symes.

The sodium salts of the two acids were employed throughout the work, and, since Ringer's solution was inadmissible as a solvent—owing to the precipitability of the α -acid by salts of calcium—were invariably dissolved in distilled water containing 0·6 or 0·9 per cent. of sodium chloride.

Three sets of observations were made with these salts:—

1. By intravascular injection, on the blood pressure and respiratory movements of cats, under full anaesthesia.
2. By perfusion on the surviving heart of the frog.
3. By hypodermic injection, on rodents and frogs, in the absence of anaesthetic.

II. OBSERVATIONS ON THE BLOOD PRESSURE AND RESPIRATORY MOVEMENTS

As already stated, these were made on cats. The animals were fully anaesthetised, and maintained in a state of complete anaesthesia by ether.

Tracings of the blood pressure were taken from the carotid artery, whilst the drug was injected into the saphenous vein.

The possibility that the salts of the α - and β -acids were, in solution, respectively pyro- and ortho-phosphonates, led at the outset to their comparison with pyro- and ortho-phosphate of sodium. The physiological action of these latter salts had been studied by Gamgee¹ in collaboration with Priestley and Larmuth, who described the pyrophosphate as being toxic to the central nervous system and to the heart, and the ortho-phosphate as inert. We have repeated and confirmed these observations.

1. Gamgee, Priestley and Larmuth, *Journal of Anatomy and Physiology*, Vol. XI, London, pp. 255-272 and 273-284, 1876-77.

Pyrophosphate of sodium

The effect of a small intravenous injection of sodium pyrophosphate (20-30 mgm. of $\text{Na}_4\text{P}_2\text{O}_7$) is to produce a temporary fall in arterial pressure without any obvious effect on the respiration. A larger dose (50-60 mgm.) produces a greater temporary fall of blood pressure with or without slowing or weakening of the respiratory movements. A lethal dose, 80 mgm. and upwards, kills either by immediate arrest of the heart,¹ followed by failure of respiration, or by immediate cessation of respiration followed by temporary recovery from the initial fall of blood pressure. As might be expected, it is the more rapid injections that favour cardiac death.

Doses of sodium orthophosphate (Na_2HPO_4) equimolecular to the above produce little or no recognisable effect either on the blood pressure or on the respiratory movements.

Solutions of $\text{Na}_4\text{P}_2\text{O}_7$ are markedly alkaline, and some of their poisonous action is due to their alkalinity. We find, e.g., that addition of hydrochloric acid up to a certain point diminishes their effect on the blood pressure, and that intravenous injections of sodium hydrate produce on the blood pressure effects closely resembling those of the pyrophosphate.

But alkalinity is not the only factor in the toxicity of these salts, for their solutions neutralised with HCl to litmus, to phenol phthalein, or to methyl orange are still poisonous, in spite of the fact that a portion of the pyrophosphate undergoes hydrolysis in the course of the neutralisation.

Orthophosphate of sodium

Gamgee, Priestley and Larmuth, employing Na_3PO_4 , found that three injections, each = 115 mgm. of this salt, produced no material change in the blood pressure or pulse rate of a rabbit weighing 1·8 kilos.

Since Na_3PO_4 must, in the blood, be transformed largely if not wholly into Na_2HPO_4 , we have preferred to use the latter salt. On a 3 kilo. cat, 142 mgm. of Na_2HPO_4 slightly raised the carotid pressure, and slowed respiration from 22 per minute to 15 per minute, whilst 284 mgm. led to a temporary fall of 40-50 mm. Hg in blood pressure with reduction of respiration from 18 per minute to 12 per minute, and 568 mgm. on the same cat produced no greater effect. These three doses

1. On one occasion after a cardiac death a clot was found in the right heart.

are, in molecules, respectively equivalent to 164, 328 and 656 mgm. of Na_3PO_4 , and to 266, 532 and 1064 mgm. of sodium pyrophosphate.

In large doses (500 mgm. and upwards), of sufficient concentration (M/2 per litre), Na_2HPO_4 leads to marked dyspnoea, commencing sometimes as late as two (or more) minutes after injection and lasting about thirty seconds. A similar effect is produced by equally hypertonic injections of other relatively inert drugs, for example, by sodium sulphate.

Sodium α -Camphene-phosphonate

Like pyrophosphate of sodium, this salt lowers blood pressure, but gram for gram, to a much smaller extent. Its effect on respiration, except in large doses, is practically zero.

In our first experiment, which was done with Professor Gamgee, the results were as follows:—

TABLE I.—Cat, 2.9 kilos, 16 March, 1909

Time	Intravenous Dose	Mean Carotid pressure	
		Before injection	After injection
3.27 p.m.	58 mgm. (2.5 c.c.)	166 mm. Hg	150 mm. Hg
3.30 "	58 " (2.5 c.c.)	154 "	150 "
3.35 "	116 " (5 c.c.)	160 "	140 "
4.2 "	249 " (5 c.c.)	160 "	128 "
4.7 "	249 " (5 c.c.)	146 "	120 "
4.46 "	131 " (5 c.c.)	132 "	118 "

As would be expected, the effects of the larger doses were greater in duration as well as in degree. There was no noticeable effect on respiration.

The three following tables show the comparative activity of the α -camphene-phosphonate, and pyrophosphate of sodium.

TABLE II. Cat A, 2.3 kilos, 24 September, 1909

Time	Intravenous dose	MEAN CAROTID PRESSURE		Respiration
		Before injection	After injection	
3.3 p.m.	Phosphonate 229 mg. (5 c.c.)	150 mm. Hg	160 mm. Hg	Unaltered
3.7 p.m.	Pyrophosphate 135 mg. (5 c.c.)	150 mm. Hg	50 mm. Hg	First quickened then slowed
3.10 p.m.	Phosphonate 229 mg. (5 c.c.)	130 mm. Hg	110 mm. Hg	Unaltered
3.13 p.m.	Pyrophosphate 135 mg. (5 c.c.)	125 mm. Hg	35 mm. Hg	First quickened, then arrested 30 sec., then recovered.

On the supposition that the camphene compound is a pyrophosphonate, these doses are equimolecular.

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TABLE III. Cat B, 2.6 kilos, 24 September, 1909.

Time	Intravenous dose	MEAN CAROTID PRESSURE		Respiration
		Before injection	After injection	
4.4 p.m.	Phosphonate 389 mgm. (8.5 c.c.)	160 mm. Hg	140 mm. Hg	Slightly slowed
4.9 p.m.	Pyrophosphate 108 mgm. (4 c.c.)	160 mm. Hg	40 mm. Hg	Frequency halved
4.50 p.m.	Phosphonate 458 mgm. (5 c.c.)	150 mm. Hg	110 mm. Hg	Slightly slowed
4.52 p.m.	Pyrophosphate 135 mgm. (5 c.c.)	150 mm. Hg	Death (heart failure)	

On the supposition that the camphene compound is a pyrophosphonate, each phosphonate dose is, in molecules, double that of the pyrophosphate immediately following it.

TABLE IV.—Cat, 2.7 kilos, 17 November, 1909

Time	Intravenous dose	MEAN CAROTID PRESSURE		Respiration	
		Before Injection	After Injection	Before injection	After injection
3.57 p.m.	Phosphonate 500 mgm. (5 c.c.)	140 mm. Hg	110 mm. Hg	26 per min.	20 per min.
4.6 p.m.	Phosphonate 1,000 mgm. (15 c.c.)	140 mm. Hg	96 mm. Hg	33 per min.	20 per min.
5.5 p.m.	Pyrophosphate 135 mgm. (5 c.c.)	114 mm. Hg	Death (heart failure)		

On the supposition that the camphene compound is a pyrophosphonate, the dose of $\text{Na}_4\text{P}_2\text{O}_7$ is equimolecular with 229 mgm. of the sodium α -camphene-phosphonate.

Sodium β -camphene-phosphonate

Intravenous injection of this salt has, on cats, effects very similar to those of the corresponding α -compound, as is seen on comparing the following table with Tables I to IV:—

TABLE V

Intravenous injections of sodium β -camphene phosphonate

Weight of cat	Dose	MEAN ARTERIAL PRESSURE		RESPIRATION	
		Before injection	After injection	Before injection	After injection
2.9 Kilos. (16 Mar., 1909)	119 mgm. (5 c.c.)	178 mm. Hg	170 mm. Hg	36 per min.	37 per min.
3.0 kilos. (24 Sept., 1909)	238 mgm. (5 c.c.)	180 mm. Hg	180 mm. Hg	37 per min.	28 per min.
	476 mgm. (10 c.c.)	180 mm. Hg	170 mm. Hg	20 per min.	11 per min.
3.4 kilos. (21 Feb., 1910)	500 mgm. (5 c.c.)	166 mm. Hg	126 mm. Hg	30 per min.	33 per min.
	1000 mgm. (10 c.c.)	162 mm. Hg	116 mm. Hg	25 per min.	26 per min.

In short, the camphene-phosphonates of sodium are, on the anaesthetised cat, relatively inert bodies. Their effects are similar and, gram for gram, approximately equal. Both, in sufficient (intravenous) dose, lower the blood pressure, and both tend to reduce the frequency of respiration.

Both are rapidly excreted in the urine, in which they are recognisable by their reaction with bromine water, about ten minutes after an injection.

III. OBSERVATIONS ON THE SURVIVING HEART

These were made on decapitated and pithed frogs. The heart was fed through the inferior vena cava or through the hepatic vein, from Marriotte flasks, with a 'head' of about 2 cm. of water. The ventricle emptied itself into the aorta, the fluid circulating through the capillaries¹ and escaping from the abdominal veins which were freely opened on the distal side of the feeding cannula. With a feeble heart it was sometimes necessary to sever one of the aortic arches.

The contractions of the heart were recorded by a light, partially counterpoised lever, connected by a thread to the apex of the ventricle.

For the reason already given, the solvent of the phosphates and phosphonates was 0.6 per cent. solution of sodium chloride in distilled water, which served also as the 'normal saline' solution against which the bodies under examination were tested.

With this 'normal' solution, oxygenated, and employed as above, a heart is usually available for experiment for upwards of one hour.

Action of sodium pyro- and ortho-phosphate

On the frog heart, administered as above, sodium pyrophosphate is markedly active. A solution containing 0.26 per cent. (M/100) may reduce the ventricle almost to standstill within one minute. As described by Gamgee and his pupils, the arrest is usually in systole,² but diastolic arrest is not uncommon. The effect of the salt can, however, be largely washed out by the normal saline. Stronger solutions act more energetically, and their effect is more permanent. A 2.6 per cent. solution (M/10) has produced permanent arrest of both chambers.

1. Apart from escape from severed arteries.
2. As with other alkaline solutions.

Sodium orthophosphate (Na_2HPO_4) is far less active. A solution containing 1·4 per cent. (M/10) reduces somewhat the force and frequency of the ventricular beat, but has much less effect than a M/100 (0·26 per cent.) solution of the pyrophosphate.

Less concentrated solutions of the orthophosphate, e.g. 0·35 per cent. (M/40) have often a restorative effect on a flagging heart, though on hearts that are beating vigorously they have usually, during perfusion, the opposite tendency.

As pointed out above, some, but not all, of the effect of sodium pyrophosphate on the anaesthetised cat is due to the alkalinity of its solutions. The same appears to be true for the surviving heart of the frog, since M/100 solutions of the pyrophosphate are more active than M/10 solutions of Na_2HPO_4 , and are not more alkaline than the latter. Owing to the gradual hydrolysis of the former salt, when its solutions are neutralised, it was not possible to investigate this point more closely.

Action of the camphene-phosphonates of sodium

The aqueous solutions of these bodies are neutral.

Gram for gram, they have on the frog's heart, as on the respiration and on the blood pressure of the cat, similar and approximately equal effects.

Dilute solutions (0·4—0·6 per cent.) of either of them first increase then slightly reduce the frequency, and usually also the amplitude, of the beat. Later the amplitude is usually increased, the frequency remaining (sometimes considerably) reduced. Weak saline solutions of camphene produce similar effects.

Solutions ten times as concentrated (4 to 6 per cent.) have failed to arrest the beat within six minutes, beyond which time lack of material has made it impossible to follow their effects.

The pyro-form, which is unquestionably assumed by the α -salt when heated, is either lost, on solution in normal saline, or (if retained) is not associated with the enhanced physiological activity that follows heating orthophosphate of sodium.

Regarding both of the phosphonates as ortho- in form, they are, in the frog's heart, molecule for molecule, more active than sodium orthophosphate, much less active than the pyrophosphate of the same metal.

IV. EFFECT OF SUBCUTANEOUS INJECTIONS OF THE PHOSPHONATES

These observations were made on frogs, rats and rabbits without anaesthesia. The α -phosphonate appeared to be the more active, but neither of them was acutely poisonous.

On frogs their effect closely resembles that of camphene. When inserted beneath the dorsal skin, this substance produces excitement followed by increasing inco-ordination and narcosis. Injections of solutions of the camphene-phosphonates lead more rapidly to the same effects, and doses of 40 mgm. of the α -compound have proved fatal within one hour.

On rats and rabbits camphene has little or no obvious effect, whether given by inhalation, or by subcutaneous injection of a solution in cod liver oil, or in ether.

The phosphonates are more active, producing effects similar to those observed on the frog.

The initial effect is excitement, accompanied by congestion of the ears, lips, genitals and pads of the feet. With sufficiently large doses this is followed by narcosis, and occasionally by death.

With both phosphonates the effects are of the same kind, but, as mentioned above, those of the α -compound seem the more pronounced, though doses of 1 mgm. per gram body weight are not invariably fatal.

V. SUMMARY AND CONCLUSIONS

It appears from the foregoing observations that the α - and β -camphene-phosphonates of sodium differ little from one another in physiological effects or in activity.

Both are far less active than the pyrophosphate, but distinctly more active than the orthophosphate of sodium.

Tested by injection, *in vivo*, they have similar and equal effects on anaesthetised cats. In the absence of anaesthesia, on rodents and on frogs, the α -phosphonate is somewhat the more active. As they have not been compared on anaesthetised rodents, or on cats in the absence of anaesthesia, it is not possible to decide whether this difference is due to the anaesthetic or to the animal.

Their effects appear to be chiefly on the central nervous system, and their comparative inertness is doubtless in part due to rapid excretion.

On the frog, and on the surviving heart of the frog, their action closely resembles that of camphene. On the warm-blooded animals the action of camphene is so much less than that of the camphene-phosphonates that comparison is difficult. Such effects, however, as are produced by camphene resemble those of minute doses of the phosphonates.

The small difference between their physiological effects, and the general resemblance of these to those of camphene, suggests that the first of the two possibilities, with regard to their constitution, quoted in the introduction, is the more probable,—namely, that they are isomeric derivatives of orthophosphoric acid, and that the pyro-form which is readily assumed by one of them (α -) on heating is as readily lost when the dried salt is re-dissolved in water.