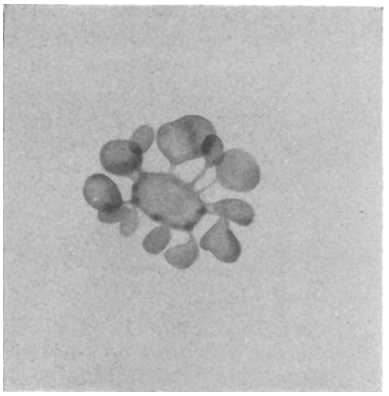

FIG. 4.
Drawing of Form 2. × 1800.

neighborhood of the implants. Form 1 (Fig. 1, 2, 3) shows a central nucleus surrounded by about 50 oval and round bodies. These small bodies have a sharp outline and an inner structure made up of vacuoles, wide pale-staining areas, and small dark granules. These bodies appear to be in the cytoplasm of a large macrophage. They resemble the larger merozoites described by Huff and Coulston.[11] Form 1 measured 21 by 12 μ.

Form 2 (Fig. 4) had a rosette appearance and showed a central core surrounded by radiating triangular or oval bodies. It measured 12 μ in diameter. It suggests a cluster of segmenters with a central core.

Discussion. These findings must be judged conservatively since (1) the forms found to date are so few, and (2) the criteria are purely morphological. The objection may be raised that in cultures of bone marrow degenerating cells are present which may be a source of error. The 2 bodies described above, however, are quite unlike the various forms taken by the degenerating cells. Moreover, their sharply-defined structures are suggestive of well-preserved animate bodies rather than of disintegrating cells. Furthermore, they resemble stages of exoerythrocytic forms in avian malaria.

Tissue culture studies are being continued in a search for additional forms in both human and simian malaria.

Addendum. After this article was submitted for publication there appeared a paper by Coulston and Huff[12] describing the exoerythrocytic forms of *Plasmodium relictum.* In the colored plates accompanying their paper are depicted forms which are similar to the 2 structures seen in the human bone-marrow. The oval bodies of the human Form 1 closely resemble the merozoites of *P. relictum* in Figs. 4, 7 and 13, while Form 2 is remarkably like the segmenter with a central core shown in Fig. 11.

11 Huff, C. G., and Coulston, F., *J. Infect. Dis.*, 1944, **75**, 231.

12 Coulston, F., and Huff, C. G., *J. Infect. Dis.*, 1947, **80**, 209.

15896

Tuberculostatic Action of Two Derivatives of the Alicyclic Compound, 3-n-Amyl-Cyclopentane-Carboxylic Acid.

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In an earlier publication it was shown that the sodium salts of certain synthetic alicyclic acids had tuberculostatic action.[1] Among the compounds tested sodium 3-n-amyl-cyclopentane-carboxylate (referred to as compound "C") was found to be among the most effective, 10 mg % being sufficient to give complete inhibition of growth of the tubercle bacillus (Strain A27) in Kirchner's medium. When the tuberculostatic action of this compound was tested by the chick chorio-allantoic technic,[2] it was found that 0.5 mg of the compound in a suspension containing

1 Emmart, E. W., *Am. Rev. Tuberc.*, 1946, **53**, 83.

2 Emmart, E. W., and Smith, M. I., *Am. Rev. Tuberc.*, 1943, **47**, 426.

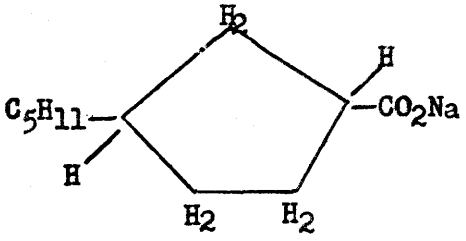
1 mg of tubercle bacilli, in doses of 1/5 cc, reduced the number of tubercles per membrane by 35%. This compound was therefore considered of sufficient interest to be used for further synthesis in the preparation of derivatives of possibly lower toxicity and higher tuberculostatic action.

Two new compounds have been prepared,* the mono-ester ("V") and the di-ester ("W") derived from sodium β-glycerophosphate and 3-n-amyl-cyclopentane-carboxylic acid.

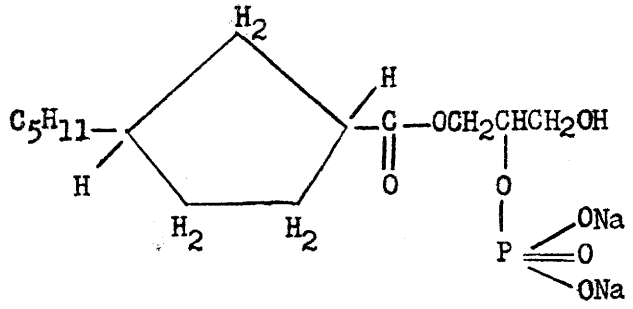
The 3 compounds referred to have the following formulae:

(Parent substance)

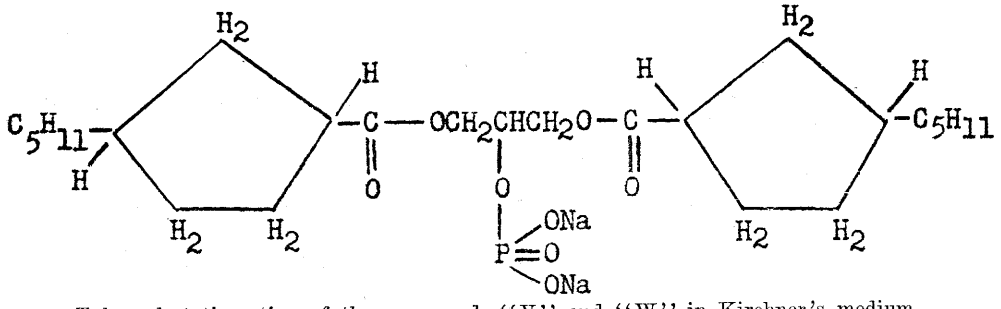
Sodium 3-n-amyl-cyclopentane-carboxylate ("C")



(Derivatives)
Mono-ester ("V")



Di-ester ("W")



Tuberculostatic action of the compounds "V" and "W" in Kirchner's medium.

In testing the tuberculostatic action of these 2 compounds dilutions of 1.2, 2.5, 5.0, 10.0 and 20.0 mg % have been used in Kirchner's medium with tubercle bacilli of the A27 human strain. The growth of the pellicle was observed and evaluated from 0 to 4, 4 representing the completely covered

* We are indebted to H. Posvic, Cornell University, for the preparation of all alicyclic compounds and their derivatives.

TABLE I.
Tuberculostatic Action of Compounds "V" and "W" *in Vitro.*

Drug	Conc., mg %	Days observed and growth of pellicle (0-4) 5	10	15	20	25	30	35
"V"	20	—	—	—	—	—	—	—
"W"	20	—	—	—	—	—	—	—
"V"	10	—	—	0.1	0.1	0.2	0.2	0.3
"W"	10	—	—	—	—	—	—	—
"V"	5	0.6	1.1	1.2	1.2	1.2	1.3	1.4
"W"	5	—	0.5	0.5	0.6	0.7	0.7	0.7
"V"	2.5	0.5	1.0	1.2	1.2	1.4	1.5	1.9
"W"	2.5	—	1.1	1.1	1.2	1.2	1.3	1.3
"V"	1.2	0.9	1.1	1.3	1.3	2.9	3.2	3.5
"W"	1.2	0.3	0.9	0.9	0.3	1.1	2.4	2.8
Control	0.0	1.1	1.3	2.1	3.9	3.9	3.9	4.0

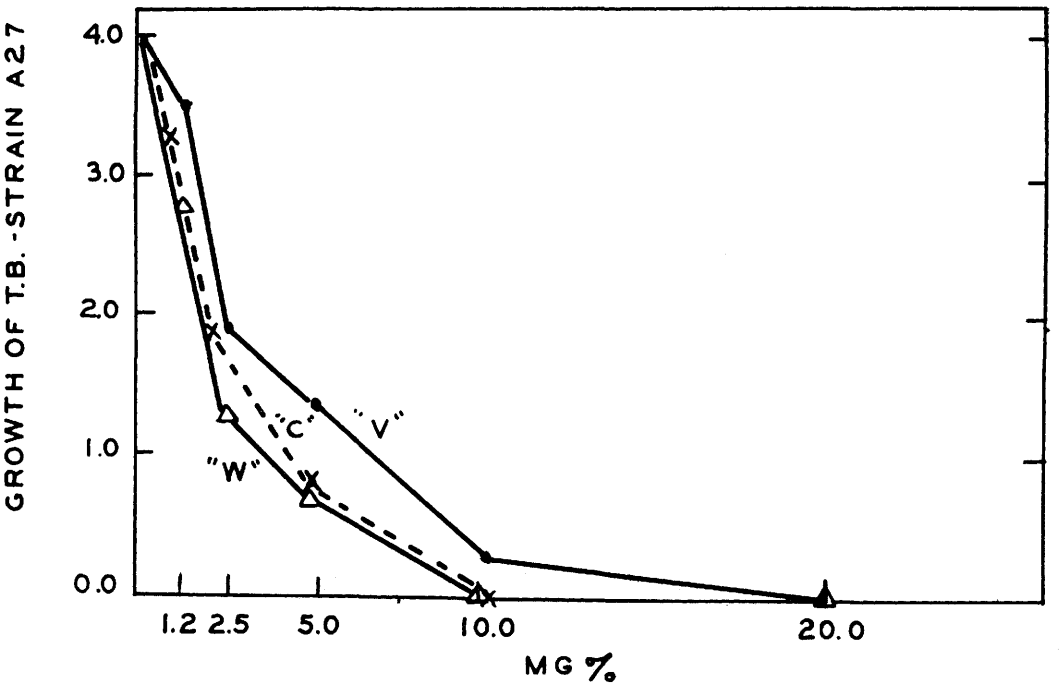


Chart 1.
Comparison of the tuberculostatic action of 3-n-amyl cyclopentane-carboxylic acid esters of β-glycerophosphate "V" and "W" with sodium 3-n-amyl-cyclopentane-carboxylate, "C," after 35 days in Kirchner's medium.

surface of the flask. Twenty mg % of compound "V" was found to give complete inhibition while 10 mg of "W" was sufficient to give similar results. Comparing the results with the other concentrations used, it was found in each dilution that compound "W," the di-ester, was more active than the mono-ester or compound "V" (Table I).

A comparison of the tuberculostatic action *in vitro* of the 2 derivatives of "V" and "W" with the parent substance sodium 3-n-amyl-cyclopentane-carboxylate indicates that there is no marked difference between the tuberculostatic action of compound "W" and "C" and both are more effective than "V" (Chart 1).

Toxicity of compound "V" and "W" as compared with the parent compound "C." In comparing the toxicity of "V" with that of "W" the effect of these compounds on the chick embryo was studied in doses of 0.6, 1.2, and 5.0 mg in 4 different experi-

TABLE II.
Toxicity of Compounds "W," "V," and "C" for Chick Embryos.

Drug	Dose, mg	No. of membranes inoculated	No. of embryos survived	% survival	% survival in relation to controls
"W"	5.0	20	0	0	
	2.5	60	9	15	35
	1.2	60	18	30	71
	0.6	20	9	45	107
	0.0 (Control)	80	34	42	
"V"	5.0	38	0	0	
	2.5	58	6	8	29
	1.2	91	22	24	88
	0.6	51	15	29	107
	0.0 (Control)	149	40	27	
"C"	4.0	30	1	3	6
	2.0	23	7	30	60
	1.0	22	8	36	72
	0.0 (Control)	48	24	50	

TABLE III.
Toxicity of the Compounds "C," "W," and "V" in White Mice.

Drug	Dose of drug in mg/20 g mouse	No. inoculated	No. survived	% mortality	Remarks
"C"	5.0	10	5	50	Survivors killed in 5 days
	7.5	11	4	64	" " "3 "
	10.0	10	0	100	All died in 1½ hr
"W"	5.0	10	9	10	Survivors killed in 5 days
	7.5	10	2	80	" " "3 "
	10.0	10	0	100	All died in 3½ hr
"V"	5.0	10	9	10	Survivors killed in 5 days
	7.5	10	4	60	" " "3 "
	10.0	10	0	100	All died in 3 hr

ments. When the data were summarized it was found that 5 mg of both compounds "V" and "W" killed all the embryos while 0.6 mg had no effect upon the per cent of embryos which survived in the control and experimental groups (Table II). Since the survival rate in the control groups varied considerably with each experiment the survival rate of each of the experimental groups was calculated on a percentage basis in comparison with the corresponding control, a value of 100 being assigned to the latter in every case. Due to its low solubility compound "C" was used in concentrations of 4.0, 2.0, and 1.0 mg. The resulting data indicate that with chick embryos no appreciable difference in toxicity of "V" and "W" can be detected (Table II).

The toxicity of the 3 drugs was also studied in white mice (N.I.H. regular strain). The compounds were injected intraperitoneally in doses of 5.0, 7.5 and 10.0 mg per 20 g body weight. Ten mice were used for each dose. Ten mg of each compound was sufficient to kill all mice in less than 4 hours, 7.5 mg of "C" permitted 36% survival for 3 days, "V"—40% and "W"—20%. Five mg of "C" gave 50% survival in 5 days, while "W" and "V" permitted 90% survival in 5 days. All 3 drugs had a narcotic effect after intraperitoneal injection. Ten mg was sufficient to produce complete narcosis in 5 minutes. Five mg affected the mice within 5 minutes but the majority recovered and appeared normal in behavior. However, a few died within 24 hours after injection.

Autopsies on the remaining animals which were sacrificed either 3 or 5 days after injec-

TABLE IV.

Tuberculostatic Action of 3-n-Amyl-Cyclopentane-Carboxylic Acid Di-ester of Sodium β-Glycerophosphate ("W") and 3-n-Amyl-Cyclopentane-Carboxylic Acid Mono-ester of Sodium β-Glycerophosphate ("V") on the Chorio-allantois.

	Dose, mg	No. of membranes inoculated	No. of embryos survived	% survival	Avg tubercles per membrane (T.B. Strain H37Rv)	Chemotherapeutic activity*
"W"	1.25	24	4	16	1.0	8.8
	0.5	58	16	27	0.9	
	0.0 (Control)	77	32	41	8.0	
"V"	1.25	30	3	10	2.0	4.6
	0.5	60	7	11	0.7	
	0.0 (Control)	60	18	30	3.2	

* Chemotherapeutic activity is the ratio obtained by dividing the average number of tubercles in the control group by that of the treated groups.

tion showed that all 3 drugs in doses of 5.0 mg produced ascites. Hemorrhages were present at the site of injection but these were less marked with "V" than with compounds "W" and "C." There appears to be little difference between the toxicity of drugs "V" and "W," however, the more rapid killing action of compounds "C" indicates that both of the compounds "V" and "W" are less toxic than the parent substance (Table III).

Comparison of the tuberculostatic action of the compounds "V" and "W" on the chorio-allantoic membrane of the chick embryo. In order to test the inhibiting effect of these compounds on the development of tubercles in the chorio-allantoic membrane of the chick embryo suspensions were prepared of tubercle bacilli of H37-Rv strain containing 1.25 mg and 0.5 mg of each compound and 1 mg of tubercle bacilli per 1/5 cc of suspension. These were incubated 24 hours at 37.5° and inoculated on the chorio-allantoic membrane of the 9-day-old chick-embryo. Since small hemorrhages were frequently seen on the surface of the membranes when doses of 1.25 mg were used the chemotherapeutic activity of the 2 compounds was calculated on a basis of 0.5 mg per membrane, a dose sufficiently low as to have no visible toxic effect. The chemotherapeutic activity is expressed as the ratio obtained by dividing the average number of tubercles obtained in the control group by that of the treated group (Table IV). Since the chemotherapeutic activity in the group containing 0.5 mg of compound "W" was almost twice that of the group containing the "V" compound it appears that the di-ester is the more effective of the 2 drugs.

Summary. 1. Twenty mg % of 3-n-amyl-cyclopentane-carboxylic acid mono-ester of sodium β-glycerophosphate ("V") was found to give complete inhibition *in vitro*, while it required only 10 mg % of 3-n-amyl-cyclopentane-carboxylic acid di-ester of sodium β-glycerophosphate ("W") to give similar results.

2. The toxicity of 3-n-amyl-cyclopentane-carboxylic acid mono- and di-esters of sodium β-glycerophosphate for the chick embryo appears to be similar to that of the parent substance, sodium 3-n-amyl-cyclopentane-carboxylate. The lethal dose for chick embryos for compounds "V" and "W" is 5.0 mg. The less soluble parent compound "C" in concentrations of 4.0 mg permitted only 3% survival.

In white mice all 3 compounds had a narcotic effect and produced hemorrhages at the site of injection but the hemorrhage was less marked when compound "V" was used. Compound "C" in doses of 10 mg per 20 g mouse had a more rapid killing effect than either "V" or "W." Five mg of "C" gave 50% mortality, while "V" and "W" in the same dose gave only 10%.

3. When the tuberculostatic effect of "V" and "W" was tested on the chorio-allantoic membrane of the chick embryo the chemotherapeutic effectiveness of "W," the di-ester, was twice as great as the mono-ester "V."