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Antipyretic Action in Rats of Tolysin Alone and in Combination with Phenacetin.

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The relative therapeutic effectiveness of tolysin has been questioned because of the fact that large quantities can be tolerated without toxic effects. This has sometimes been attributed to inefficient absorption. Sufficient evidence has now accumulated to show that tolysin is absorbed in full therapeutic doses in humans, dogs and rats.¹⁻⁴ To date, however, the margin existing between therapeutic dosage and the limit of absorption has never been clearly demonstrated. Previous investigation has shown that 1,000 Mg./K. of tolysin, when given to rats daily by oral administration was completely absorbed and non-toxic, as evidenced by normal growth rate, absence of symptoms or autopsy findings.⁴ The therapeutic response to 25% of this dose is depicted in the following:

Antipyresis in rats was used as the basis for the evaluation of drug action. The animals were febrile by the subcutaneous injection of yeast, using the technique described by Smith and Hambourger.⁵ This results in a fever maintained at a fairly constant high level for over 30 hours. Such a lasting fever is a necessity in the study of antipyretics characterized by delayed onset and long duration of action.

During experimentation, the rats were kept in a constant temperature room at 86°F. Drugs were administered by stomach tube, in an acacia suspension, 18 hours after the removal of food. A single experiment consisted in temperature measurements not only on 4 rats for each drug given, but also on 4 fever controls, and 4 room controls. The antipyretic effects recorded are corrected for variations noted in the 2 control groups.

The antipyretic effect of tolysin on febrile rats receiving 250

¹ Barbour, H. G., Lozinsky, E., and Clements, C., *Am. J. Med. Sci.*, 1923, **105**, 708.

² Fuerth, O., and Kuh, E., *J. Pharmacol. and Exp. Therap.*, 1930, **38**, 57.

³ Barbour, H. G., and Lozinsky, E., *J. Lab. and Clin. Med.*, 1923, **8**, 217.

⁴ Barbour, H. G., and Gilman, A., *J. Pharmacol. and Exp. Therap.*, 1935, **55**, 400.

⁵ Smith, P. K., and Hambourger, W. E., *J. Pharmacol. and Exp. Therap.*, 1935, **54**, 346.

Mg./K. of the drug is depicted in Fig. 1. This action differs from that of most analgesic-antipyretic drugs in that the onset of action is delayed for 2 hours, whereas its duration is prolonged for over 8 hours. From the 2°F. temperature fall noted, it is obvious that $\frac{1}{4}$ of the amount of tolysin that can be easily absorbed by rats following a single oral administration has marked therapeutic action.

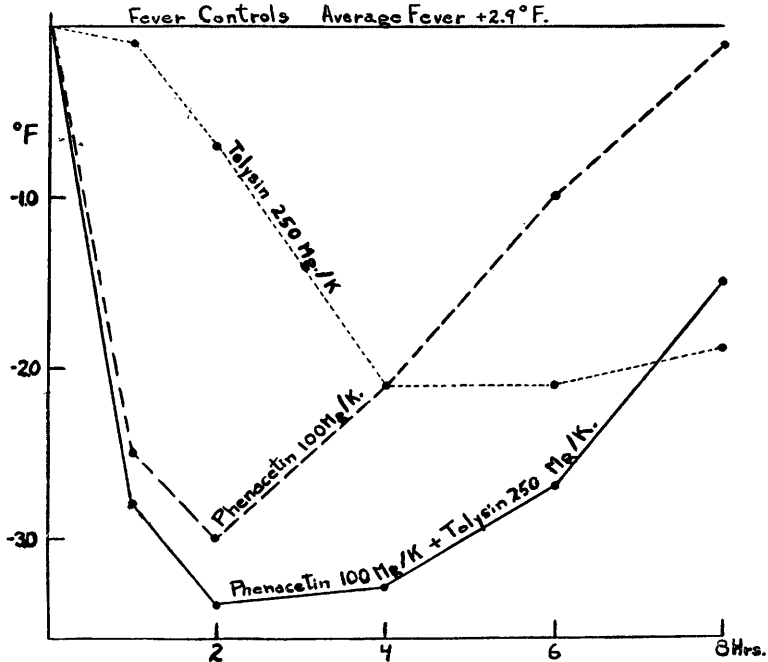


FIG. 1.

The antipyretic action of tolysin, phenacetin, and their combination.

The analgesic-antipyretic drugs commonly used are characterized by prompt action, but are handicapped by short duration of activity. In view of the nature of tolysin antipyresis as regards delayed onset and prolonged effect, combination with a quicker acting drug should result in rapid onset and prolonged duration of action.

Using the technique described above, phenacetin (100 Mg./K.) alone, or in combination with tolysin (250 Mg./K.) was given orally to fevered rats. Figure 1 shows the average results obtained on 56 animals, divided equally between phenacetin alone and the combination of phenacetin plus tolysin.

It can readily be seen that the maximum effect of phenacetin is obtained almost immediately and rapidly wanes after 2 hours. The antipyresis from tolysin is obtained more slowly and tends to be

more lasting. The most pronounced effect from tolysin is not observed until 4 hours after administration and continues practically unabated for 4 more hours. The results obtained by combined administration, as would be expected, are additive. Maximal effects are obtained at a time when both drugs are active. However, effective tolysin antipyresis persists after that of phenacetin, giving a sustained and powerful action.

TABLE I.
Toxicity of Phenacetin, Tolysin, and Phenacetin and Tolysin.

Drug	Dose G./K.	No. Rats	No. Survivals		% Survival	
			24 Hr.	48 Hr.	24 Hr.	48 Hr.
Tolysin	15	8	8	8	100	100
Phenacetin	6	8	0	0	0	0
Tolysin	15	8	5	4	67½	50
+	+					
Phenacetin	6	15	2	1	13	7
Phenacetin	4					
Tolysin	10	15	8	2	57	13
+	+					
Phenacetin	4	15	15	11	100	73
Phenacetin	2					
Tolysin	5	15	15	11	100	73
+	+					
Phenacetin	2					

In a combination of drugs, it is necessary to determine to what extent the toxicity of the more toxic component may be enhanced by the other drug. In the present instance both drugs are relatively benign. As can be seen from Table I, tolysin is non-toxic to rats in doses as high as 15 G./Kilo, probably because of the limits of absorption. Phenacetin, the more toxic element of the combination, when administered orally has a minimal lethal dose (50% fatality) between 2 and 4 G./K. Combinations of tolysin and phenacetin in a ratio of 2½:1, are no more toxic per given amount of phenacetin than phenacetin alone. When the amount of phenacetin in such a combination exceeds the m.l.d., the presence of so much tolysin even proves protective. This protection is probably the result of a mechanical effect in the gut, large amounts of tolysin preventing the rapid absorption of phenacetin. When given in small doses, however, as shown above, the presence of tolysin in no way interferes with the absorption and antipyretic action of phenacetin.

1. Tolysin in doses of 250 Mg./K. exhibits in fevered rats a marked antipyretic action of slow onset, persisting for more than 8 hours. This amount represents less than 25% of the easily absorbable dose previously found by us.³

2. When this dose of tolysin is combined with phenacetin (100

Mg./K.) antipyresis of both rapid onset and long duration is obtained.

3. The presence of tolysin in no way augments the toxicity of phenacetin in rats.

8476 C

Permeability of Red Cell Membrane after Hypotonic Hemolysis.

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The generally accepted explanation for hypotonic hemolysis is that the red cells take up water, swell, and finally become permeable because their membranes are stretched or under tension. Conductivity measurements, however, show that the resistance of a hemolysed suspension is such that the cell membranes must have a very low conductance (Fricke and Curtis¹), an observation which forces one to consider the possibility that the increased permeability associated with lysis is temporary only, and followed by a more or less complete repair of the membrane.

1. The simplest evidence is to be obtained from systems in which "reversible hemolysis" occurs. This phenomenon is observed when several volumes of water are added to blood or washed cells so as to produce more or less complete lysis, and enough hypertonic NaCl to restore the original tonicity added subsequently. The opacity which appears on the restoration of the tonicity was thought by Brinkman and Szent-Gyorgi² to be due to the taking up of liberated hemoglobin on or in the cells, but Bayliss³ showed that it is the result of two occurrences: (a) if the amount of added water is small, swollen but unhemolysed cells shrink to their original size on the re-establishment of the tonicity, and (b), if the amount of added water is great, hemolysed ghosts shrink under the same conditions, and, trapping hemoglobin, bring about optical heterogeneity.

If to 10 cc. of a washed rabbit red cell suspension with a volume concentration of about 0.7 is added 40 cc. of water, a tonicity in the neighborhood of 0.2 is established, and this is low enough to hemolyse virtually all the cells, as can be shown by the hemoglobin con-

¹ Fricke, H., and Curtis, H. J., *J. Gen. Physiol.*, 1935, **18**, 821.

² Brinkman, R., and Szent-Gyorgi, A., *J. Physiol.*, 1923, **58**, 204.

³ Bayliss, L. E., *J. Physiol.*, 1924, **59**, 48.