

58 (314)

**A study of "protagon" prepared by the Wilson-Cramer method.**By **L. J. COHEN** and **WILLIAM J. GIES.**

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Two months ago we received a copy of the January issue of the journal containing Wilson and Cramer's paper on "Protagon."<sup>1</sup> Results recently published from this laboratory<sup>2</sup> and also from Halliburton's,<sup>3</sup> appeared to prove conclusively that protagon is always merely a mixture of substances and that it has no constant chemical qualities because of its invariable mechanical heterogeneity. Wilson and Cramer view our data from another standpoint, however. They have presented a few results which they believe completely invalidate our prior conclusions against the chemical individuality of protagon. We cannot agree with them in this opinion and are surprised to find their paper so weak in support of the far-reaching deductions they summarize on its concluding page.

Numerous inconsistencies in the paper by Wilson and Cramer must be frankly considered if the truth regarding protagon shall prevail. In this preliminary communication, however, we shall refer chiefly to the revival of the old and frequently abandoned notion<sup>4</sup> that treatment of protagon with warm alcohol effects its *chemical decomposition* which, they say (p. 105), "has not been suspected previously."

Regarding the action of warm alcohol on protagon, Wilson and Cramer say: "The statement made in a former paper by Cramer, that protagon is not decomposed by warm ether or boiling alcohol, must therefore be corrected. *In the case of boiling alcohol it is true only if the solvent is prevented from acting on*

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<sup>1</sup> Wilson and Cramer: *Journal of Experimental Physiology*, 1908, i, p. 97.

<sup>2</sup> Lesem and Gies: *American Journal of Physiology*, 1902, viii, p. 183; Gies and collaborators: *Biochemical Researches*, 1903, i, p. 263 (reprint no. 11); Posner and Gies: *Journal of Biological Chemistry*, 1905, i, p. 59; Gies: The same, 1906, ii, p. 350; Steel and Gies: *American Journal of Physiology*, 1907, xx, p. 378.

<sup>3</sup> Rosenheim and Tebb: *Journal of Physiology*, 1907, xxxvi, p. 1.

<sup>4</sup> Gies: *Journal of Biological Chemistry*, 1907, iii, p. 350.

*protagon for some time.*"<sup>1</sup> Wilson and Cramer attribute the hypothetical decomposing effect of warm alcohol on protagon to an assumed "hydrolyzing (!) action." They present no facts, however, that justify this guess.

Wilson and Cramer have prepared protagon by a new method, in which the brain tissue is extracted first with cold 96 per cent. alcohol, later with cold ether, for the complete removal of cholesterol and lecithin. The brain mass remaining after this treatment is then pulverized and extracted with boiling absolute alcohol. "The boiling solvent is poured on the powder and the mixture kept boiling for one or two minutes in a warm bath, moving the mixture all the time."<sup>2</sup> The alcoholic solution is filtered through a hot-water funnel; the filtrate is allowed to drop into a vessel cooled in ice. The same process of extraction is repeated twice. The crude crystalline product is washed with ether and dried in vacuo. Recrystallization is effected by pouring boiling absolute alcohol on the sample of protagon. The solution is kept boiling for one minute and then filtered as before."

The protagon products prepared by Wilson and Cramer by this method contained between 0.9 per cent. and 1.0 per cent. of phosphorus, after the fourth or fifth recrystallization. The general composition of their preparations is about the same as that of the products previously made by Cramer<sup>3</sup> by a method which, they assume, as quoted above, involved decomposition and *the ultimate isolation, therefore, of a mixture.*

Wilson and Cramer refer with great satisfaction to the "constancy of the chemical composition" of their products as something very striking and important, but, as they fail to give such details of their treatment as the mutual proportions of the protagon and the solvent employed in their recrystallizations, we are not at all impressed by the analytic constancy referred to.

As was stated above, Wilson and Cramer believe that Gies and his collaborators unknowingly subjected their protagonists to *decomposition* by the "hydrolyzing action" of the warm alcohol used in the fractional recrystallization experiments that yielded the re-

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<sup>1</sup> Italics our own.

<sup>2</sup> Italics our own.

<sup>3</sup> Cramer: *Journal of Physiology*, 1904, xxxi, p. 31.

sults which have lately made protagon appear to be a mechanical mixture. It is very noticeable, however, that Wilson and Cramer, in spite of their insistence upon this conclusion, have failed to give their opinion a thorough test. They have not ascertained whether fractional recrystallization of protagon by their own non-decomposition method does not yield results similar to those they ascribe to hydrolytic decomposition in the experiments in this laboratory. They declared that treatment with boiling absolute alcohol, for from one to two minutes at a time by their new method, does not bring about such hydrolytic decomposition. Then why did they not show that fractional recrystallization, by their non-decomposition method, yields fractions of identical composition?

We have endeavored to settle the doubt in this matter on the ground selected by Wilson and Cramer. We have prepared protagon by carefully following every detail of their very simple method, as given by them. We cannot believe that defects in our technique have accounted for any of our results. Nevertheless, we obtained protagon which *steadily decreased in phosphorus content each time it was recrystallized by the Wilson and Cramer method* (1.3 per cent. to 0.4 per cent. in four recrystallizations); which never completely redissolved in the moderate excess of boiling absolute alcohol we used for the purpose; which, on recrystallization, each time left in the mother liquor material having a much higher phosphorus content than the corresponding crystallized portion; and which, on fractional division by recrystallization at 20°C. and 0°C., yielded two crystalline products, and material in the final mother liquor, that were *strikingly unlike, physically and chemically*. In short, simple fractional recrystallization of protagon made by the Wilson-Cramer method, in which treatment, according to Wilson and Cramer, *decomposition does not occur*, has given results which confirm conclusively the data and deductions published by Gies and his collaborators, that Wilson and Cramer have sought unsuccessfully to explain away.

Rosenheim and Tebb<sup>1</sup> also recently examined Wilson and Cramer's results, but have not been able to confirm Wilson and Cramer's conclusions.

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<sup>1</sup> Rosenheim and Tebb: Proceedings of the Physiological Society, *Journal of Physiology*, 1908, xxxvii.

In a more detailed publication of our results we shall shortly draw attention to the fact that if certain of the statements made by Wilson and Cramer are correct, Gamgee's protagon cannot be regarded as a definite chemical compound, but must be accepted as a mixture, quite unlike the product that Wilson and Cramer now designate as protagon. Wilson and Cramer consider Gamgee's protagon identical with their own and the standard product for all protagon comparisons. They also appear to believe that anything having the quantitative elementary composition of Gamgee's protagon, whatever the method employed to prepare it, is protagon.