ON THE CHARACTERISATION OF DIFFERENT AMYLASES.

By K. Venkata Giri.

Various methods have been suggested for characterising the different amylases occurring in plants and animals.

Kuhn (*Annalen*, 1925, **444**, 1) differentiated diastatic enzymes into two groups, the α and the β amylases, which attack different points of the starch molecule. Studies on the mutarotation of the sugars formed upon degradation of starch by amylases have led to the discovery that α amylases produce α maltose as the end product whereas the action of β amylases results in the primary formation of β maltose. Kuhn has shown by this method that pancreas and taka-diastase belong to the group of α amylases, while malt amylase can be regarded as a member of the β group. According to Ohlsson and his co-workers (*Compt. rend. Soc. Biol.*, 1922, **87**, 1183; *Compt. rend. Trav. Lab. Carlsberg*, 1926, No. 7, 16; Nordh and Ohlsson, *Z. Physiol. Chem.*, 1931–32, **204**, 89; Ohlsson and Uddenberg, *Ibid.*, 1933, **221**, 165), the dextrinogen amylase (α amylase) and the saccharogen amylase (β amylase) are differentiated by the relative hydrolysis of starch as determined by changes in iodine colouration and reducing action. Waldschmidt-Leitz and Reichel (cited from Willstätter and Rohdewald, *Z. Physiol. Chem.*, 1933, **221**, 13) have developed a method which consists in determining the velocity coefficients of sugar formation and iodine colouration. It has been shown by this method that α amylase of malt and β amylase of barley form 11 and 103 per cents. respectively of maltose from amyloamylose, at which stage the colouration with iodine is negative. This method has been followed by Willstätter and Rohdewald (*loc. cit.*) in their researches on leucocyte amylases wherein they have shown that the amylase is a dextrinase.

The present communication relates to a simple qualitative method of characterisation and differentiation of amylases. In principle the method is based on the observations of Wijsman (*Rec. Trav. Chim.*, 1890, **9**, 1) who was the first to postulate the two enzyme theory of malt amylase in a definite form. He carried out some diffusion experiments with malt amylase and concluded that diastase is a mixture of two enzymes. Later on, several workers have definitely established the existence of the two components of malt amylase. As for the other amylases no definite experimental evidence has so far been adduced to show their plural nature. In the present communication some fresh

evidence has been obtained to indicate the plural nature of some of the amylases investigated.

EXPERIMENTAL.

The method briefly consists in adding a small drop of enzyme solution to a thin layer of agar gel impregnated with starch in a petri-dish and allowing it to diffuse for 28 to 48 hrs. at the laboratory temperature. At the end of the period a dilute solution (N/200) of iodine is poured on the plate, and allowed to remain there for about 2–3 mins., until the colours of all the diffusion zones come out clearly. A deep blue background with a round diffusion field at the centre is formed. The central diffusion field is coloured violet in the case of β amylase and colourless in the case of α amylase. Amylases which contain mixtures of both the components produce a central colourless diffusion field with a violet or green and violet zones surrounding it, depending on the nature of the amylase and starch used.

The substrate medium for the diffusion experiments is prepared as follows: A gel containing 1 per cent. agar-agar together with 0·5 per cent. of soluble starch or 0·2 per cent. of potato starch is prepared. It is then melted and distributed into a number of petri-dishes each of about 4 inches diameter and after the mixture is set, a small drop of the enzyme solution is added to the agar-agar plate, and the diffusion allowed to proceed at the laboratory temperature. Usually, a 24-hour period is chosen for diffusion. If more time is allowed, the diffusion zones become broadened, and clearer and better defined zones are formed.

Figs. I, II and III are reproductions of the diffusion zones obtained for different amylases—(1) Barley malt amylase. (2) β amylase of barley malt (prepared according to Ohlsson's method). (3) Sweet potato amylase (prepared according to the method described by the author (*J. Indian Chem. Soc.*, 1934, **11**, 339). (4) Pancreatic amylase (B. D. H.). (5) Taka-diastase (Parke, Davis & Co.). (6) Salivary amylase.

Figs. I and II show diffusion zones obtained with soluble starch (Zulkowsky's) as substrate, while Fig. III shows the diffusion zones of pancreatic and salivary amylases and taka-diastase with potato starch (B. D. H.) as substrate.

Fig. I is a reproduction of the diffusion zones obtained with malt amylase (*a*), β amylase from malt (*b*) and sweet potato amylase (*c*). It can be seen that sweet potato amylase gives a violet coloured diffusion field similar to that of β malt amylase thereby showing that it is a β or saccharogen amylase. On the other hand, malt amylase which consists of both α and β components gives two diffusion zones, a central one which is colourless and a violet coloured diffusion zone surrounding it.

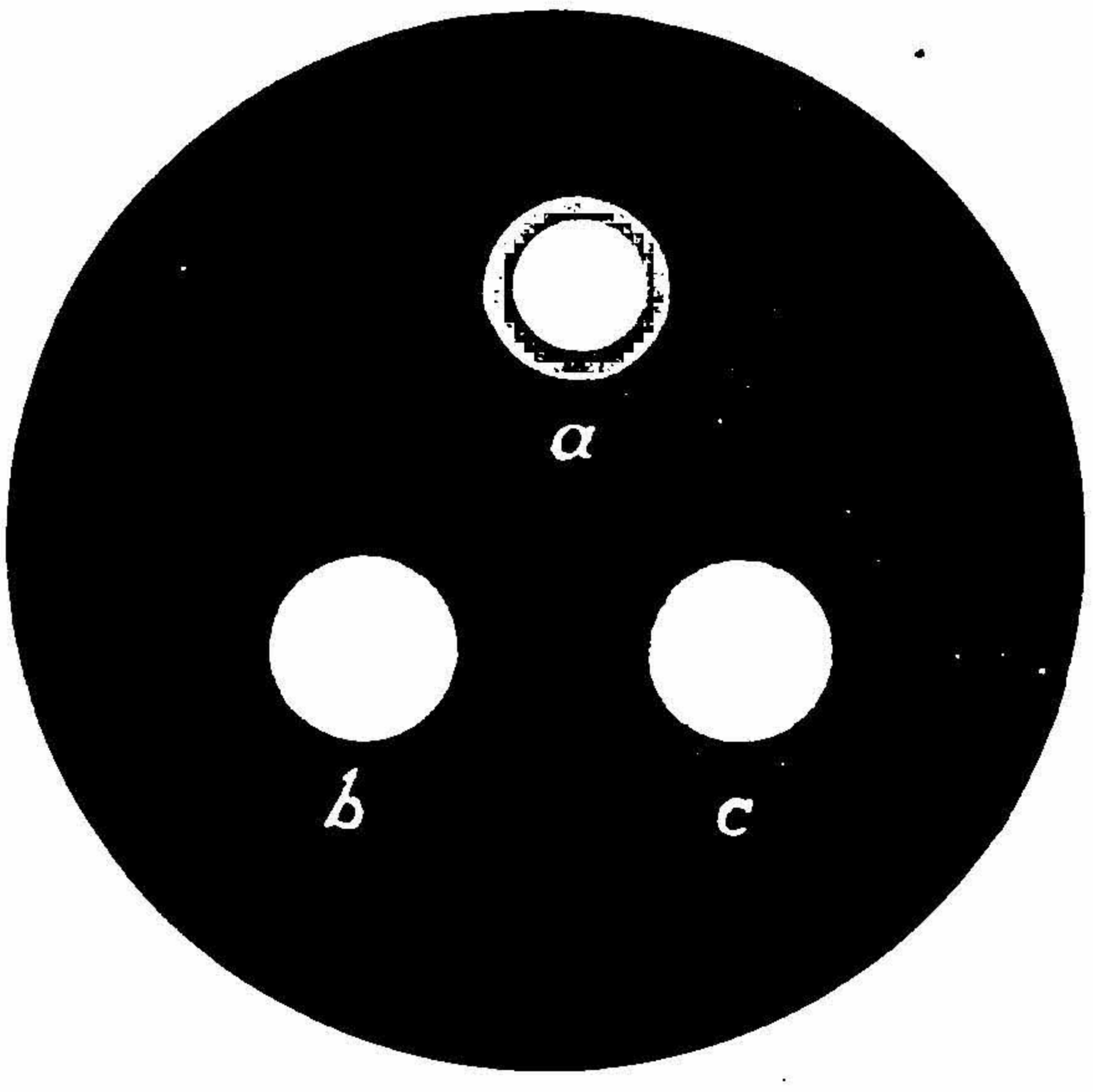

Fig. I.

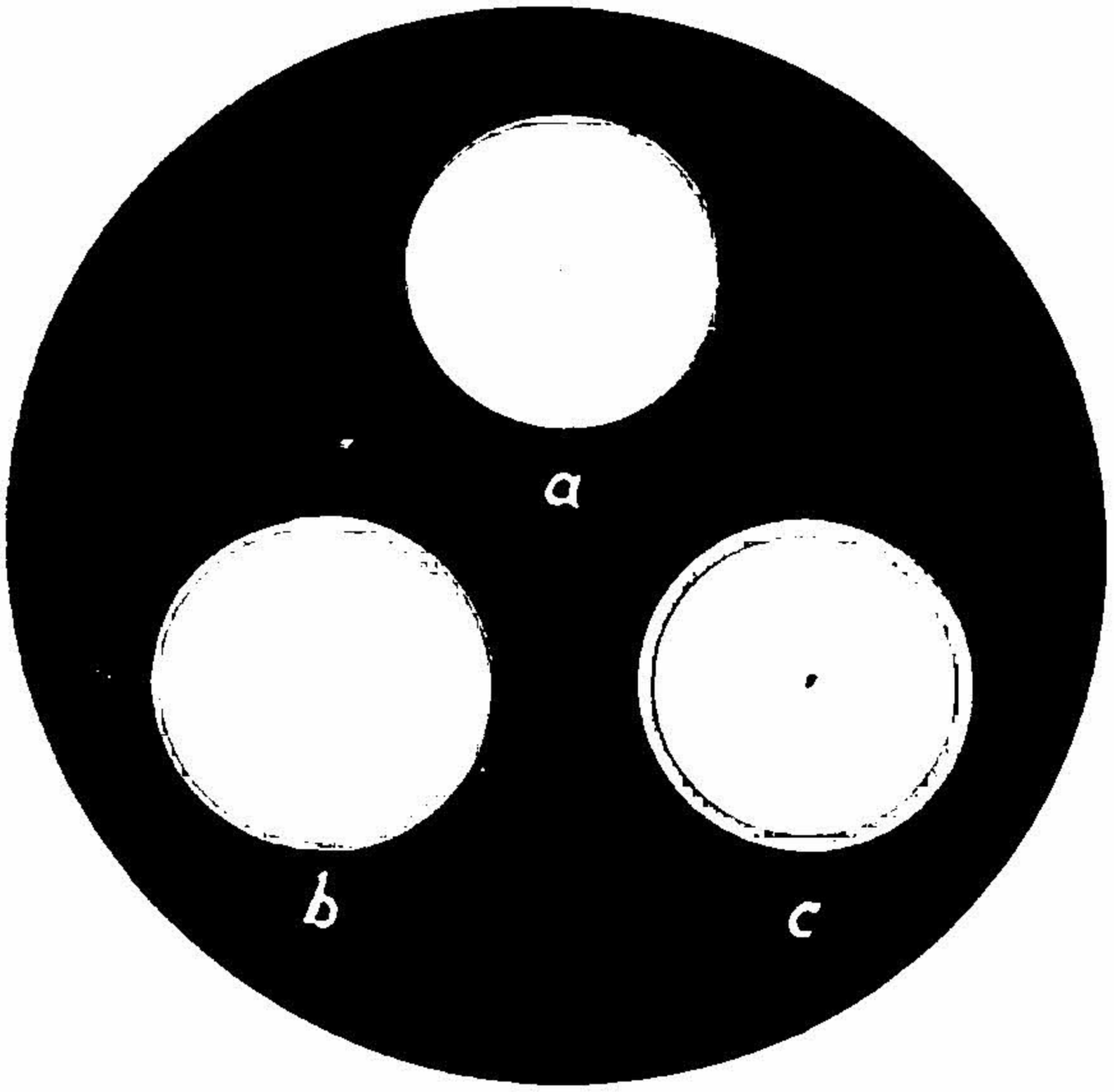

Fig. II.

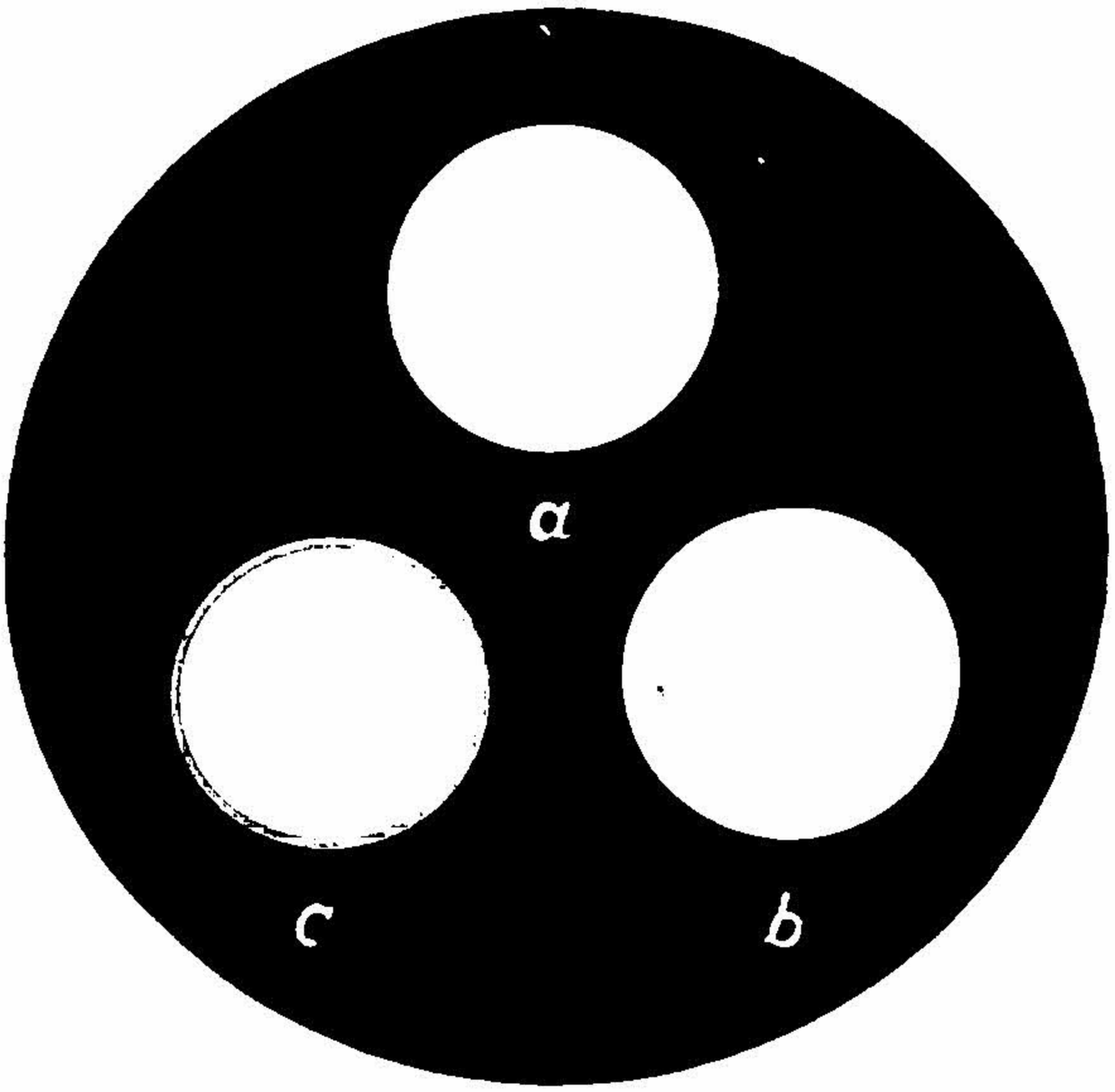

Fig. III.

Thus, amylases containing both components can be easily differentiated from those which contain mainly one component.

Fig. II represents the diffusion zones obtained by salivary amylase (*a*), pancreatic amylase (*b*) and taka-diastase (*c*). Here again there is a distinct difference between the diffusion zones obtained for the three amylases. In the case of taka-diastase there are three distinct diffusion zones—a central, colourless field, a broad green zone, and finally a distinct violet streak surrounding them. In the case of pancreatic amylase, the green coloured zone is narrow compared with that of taka-diastase. With salivary amylase all these characteristics are developed to a far less extent than with the other two enzymes. In all the three cases, however, there appears to be at least two clear diffusion zones, which suggest the presence of two different components.

The differences are more clearly brought out in Fig. III, which reproduces the diffusion zones obtained by the amylases when potato starch is used as substrate. Thus the diffusion zones obtained for taka-diastase are three in number—a colourless central field, a broad, green coloured zone, and finally a very distinct and deep violet coloured ring at the fringe. In the case of pancreatic and salivary amylases, however, only two diffusion zones are formed—a central colourless zone and a green coloured one surrounding it. There is gradation in size and width of the green coloured diffusion zone in all the three cases, taka-diastase producing a broader diffusion zone than pancreatic amylase. In the case of salivary amylase the green coloured diffusion zone is scarcely visible. The three distinct diffusion zones formed by taka-diastase is a characteristic feature indicating the plural nature of this amylase. Attempts to separate the components by physical or chemical methods are in progress.

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Note.—After the work described in the present paper was completed, there appeared a communication by Giesberger (*Proc. Kon. Akad. Wetensch. Amsterdam*, 1934, **37**, 188) in which the author concludes from diffusion experiments through gelatin that pancreatic and salivary amylases produce diffusion zones which are characteristic of the component nature of the amylases, thereby confirming the observations made independently by the author. The method of Giesberger suffers, however, from the disadvantage that when gelatin is used as diffusion medium, it is liquefied particularly when taka-diastase is used, with the result that all the diffusion zones are not clearly visible—hence the superiority of the present method to that of Giesberger.