

Fig. 3
a b and c, with 0.1 per cent trypsin

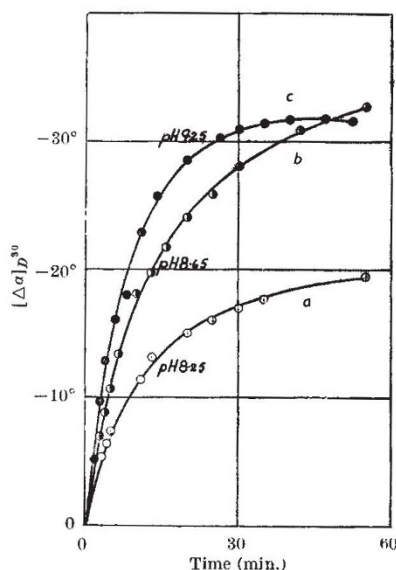


Fig. 4
a b and c with 0.1 per cent trypsin

The splitting of native β -lactoglobulin (in systems without urea) is accompanied by an increase in lævo-rotation (see, for example, curve *a*, Fig. 4), and according to the preceding paragraph it seems natural to regard this as an indication that the process of denaturation (with a large increase in rotation) precedes the splitting of peptide bonds (accompanied by a relatively small decrease in lævo-rotation).

In order to elucidate this further and to see if, under these conditions, there is a similar interaction between the enzyme and the alkaline environment, the stability of β -lactoglobulin in this medium was investigated. At pH 8.25 a slow denaturation was found (measured by increasing lævo-rotation and insolubility in the salt-buffer mixture previously described), and the speed was found to increase with rising pH. Curve *a*, Fig. 2, shows this alkali denaturation at pH 9.25, and by analogy with Fig. 1 it is further shown how increasing concentrations of trypsin catalyse this process. Control experiments with heat-treated trypsin (0.01 per cent) gave a curve exactly like curve *a*.

The dotted curve in Fig. 2 shows the percentage of total protein precipitated at different times by the salt-buffer mixture; the curve has been constructed from analysis of samples removed at intervals from a system corresponding to curve *c* of Fig. 2 (0.01 per cent trypsin). The curve gives the direct proof that, under these conditions, more denatured protein is produced than the enzyme can break down immediately to unprecipitable products.

Fig. 3 shows how the rate of splitting increases with rising pH values (three values given). On the basis of the experiments described above, the probable explanation for this is that the pure alkali denaturation, and consequently the alkali denaturation catalysed by trypsin, proceeds more quickly as the medium becomes more alkaline (Fig. 4).

According to Northrop⁴, the degree of ionization of the protein substrate determines the optimum pH for splitting, an explanation which Bergmann and Fruton⁵ from their investigations on the breakdown of synthetic substrates have described as inadequate.

In the case of β -lactoglobulin, alkali denaturation will, of course be of great importance in determining the optimum pH (this will naturally also be dependent on the stability of the trypsin and as additional experiments seem to show, among other things also on the concentration of the trypsin and on the temperature chosen). The observation⁶ that the optimum pH for the trypsin splitting of denatured protein is lower than for breakdown of the same in the native state may support the view given above. So far it is uncertain if alkali and acid stability have the same importance for the proteolytic hydrolysis of other native proteins.

It was thought to be interesting to compare β -lactoglobulin with trypsin-resistant proteins such as native serum albumin and egg albumin, which

are easily split in the denatured state. In contrast to lactoglobulin, these are not easily denatured by alkali (standing several days at pH 9). Neither was trypsin found to have any catalytic effect on the denaturation of egg albumin in a weakly denaturing system (25 per cent urea, pH 9.25), and both conditions for splitting were thus missing here.

The results given here are in agreement with the hypothesis proposed by Linderstrøm-Lang *et al.* for denaturation as the initial process in trypsin splitting. They demonstrate, however, that the mechanism of this process is more complicated than these investigators had supposed, and a further analysis cannot be given before additional experimental material is available. I am indebted to Prof. Linderstrøm-Lang for valuable criticism.

¹ Linderstrøm-Lang, K., Hotchkiss, R. D., and Johansen, G., *Nature*, **142**, 996 (1938).

² Lundgren, H. P., *J. Biol. Chem.*, **138**, 293 (1941).

³ Jacobsen, C. F., and Korsgaard Christensen, L., *Nature*, **161**, 30 (1948).

⁴ Northrop, J. H., *J. Gen. Physiol.*, **5**, 263 (1922).

⁵ Bergmann, M., and Fruton, J. S., "Advanc. Enzymol.", **1**, 63 (1941).

⁶ Oppenheimer, C., *Die Fermente*, Supp. **1**, 637 (1936).

NUCLEAR INTERACTIONS OF THE PARTICLES PRODUCED IN COSMIC RAY BURSTS

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WE wish to report the preliminary results of an investigation to study nuclear bursts, carried out recently with a counter-controlled cloud chamber at the Laboratorio della Testa Grigia (3,500 m.).

By a 'nuclear burst' we mean any event in which a particle (ionizing or not), by interacting with a nucleus, gives rise to new ionizing particles, generally different in nature and in energy. In this definition

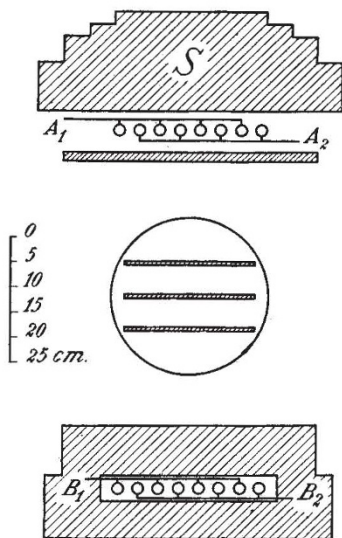


Fig. 1

unlike events such as stars, penetrating showers, mixed showers, etc., are included¹.

The arrangement used is represented in Fig. 1. The cloud chamber expanded whenever one counter at least was discharged in each of the groups A_1 , A_2 , B_1 , B_2 , and for this two particles at least were necessary. The cloud chamber was 32 cm. in diameter with an observable depth of 10 cm. It contained three 1.2-cm. lead plates, and was filled with argon at about 85 cm. pressure. Stereoscopic photographs were taken.

In 205 hours, 801 photographs were obtained, from which we selected those which appeared interesting owing to the presence in the cloud chamber of: (a) groups of particles originating from a common point in the lead S (Fig. 1), or in the three plates inside the chamber, and in which penetrating particles (recognizable as not generating secondaries in any plate) or heavy ionizing ones were present; (b) showers with no evidence of penetrating particles but certainly descending from S , so that the showers appeared under 23 cm. of lead (referred to as electron showers).

Thirty-eight photographs present showers of penetrating particles only (penetrating showers), produced in the lead S ; nine mixed showers, that is, showers that certainly contain electrons and penetrating particles; sixteen electron showers.

In twenty-two photographs, nuclear bursts appeared produced in the lead plates of the cloud chamber associated or not (thirteen and nine respectively) with local showers produced in S and recognizable in the cloud chamber. Six of thirteen associated bursts clearly show the penetrating particle producing them.

The study of the properties of the penetrating particles of the bursts in regard to the production of new bursts and to the scattering permits us to formulate some conclusions.

Successive multiple production. Penetrating showers observed by us are nearly all produced in the deeper region of S . Moreover, we observe that the penetrating particles of a penetrating shower seen in the cloud chamber are normally not the same as those which have discharged A_1 and A_2 . This is in accord with the hypothesis that the energetic bursts which

give rise to the so-called penetrating showers generally contain a large number of short-range particles, too. This agrees with a recent suggestion of Powell and co-workers², and confirms that the penetrating showers and the bursts observed in the cloud chamber are of the same fundamental structure, eventually differing only in energy. Moreover, burst production has been found to be the principal interaction of the penetrating particles of the local showers produced in S , with the plates inside the cloud chamber.

Therefore, we are led to a sort of cascade representation³, in which the most energetic of the particles produced may successively give rise to new bursts. We can make an attempt to clear up this point with the following estimate. In the 46 penetrating and mixed showers observed, we counted 142 particles which traversed the 1.2-cm. lead plates inside the cloud chamber a total of 380 times; six penetrating particles of these showers produced a new burst, with heavy ionizing and penetrating particles, in a plate of the cloud chamber. Hence we obtained a mean cross-section for the production of new bursts:

$$\sigma = \frac{6}{380 \times 1.2 \times n} \frac{1}{p} = \frac{4 \times 10^{-25}}{p} \text{ cm.}^2 \text{ per nucleus,}$$

where n is the number of lead nuclei per cm.³, and p is a mean probability that a burst produced within the plate may still appear in the cloud chamber with heavily ionizing particles.

The value of p is uncertain, for its average value has certainly to be extended to very wide energy limits. On the basis of recent work^{4,5}, we have estimated that a value $p \sim 0.4$ may be suitable. With this value we obtain $\sigma \sim 10^{-24}$ cm.². It is interesting to note that this value of σ is not far from the geometrical cross-section of the lead nucleus (2.5×10^{-24} cm.²), and is near the value already established for the particles of the total component which produces bursts⁶.

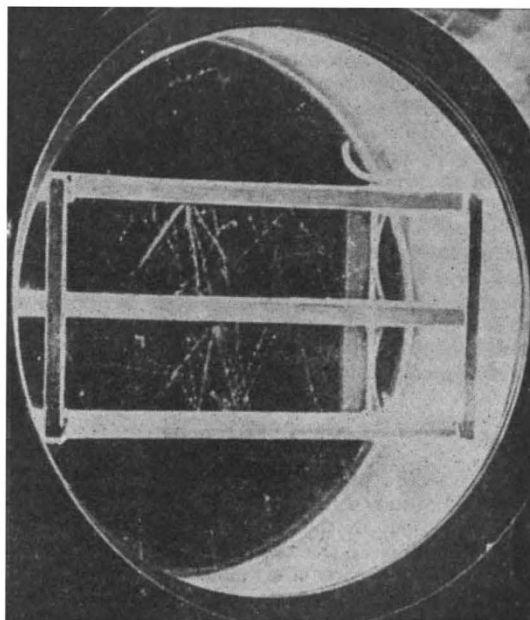


Fig. 2

Our results show, moreover, that, in the absorption measurements of the radiation which produces nuclear disintegrations⁴, a large percentage of the events observed (stars and bursts) are produced by the secondaries generated in the absorbers.

Scattering. We have searched for other interactions of the penetrating particles of the bursts, that is, for scattering. To do this we measured the scattering angles through the three plates of thirty-eight penetrating particles associated in bursts produced in *S* in a manner similar to that used by Rochester⁷. These tracks were found on photographs selected *a priori* owing to their being technically good. The scattering observed is never larger than 6°, except in one case (9°), and can be explained by Coulomb scattering alone, without any anomalous scattering. One scattering of ~25°, on the contrary, has been observed in penetrating particles not clearly associated in bursts.

A typical photograph of a burst produced by a penetrating particle of a penetrating shower is reproduced in Fig. 2.

A detailed report will be submitted to the *Nuovo Cimento*. We are greatly indebted to Prof. B. Ferretti for helpful discussions.

¹ Rossi, B., *Rev. Mod. Phys.*, **20**, 537 (1948), and references cited there.

² Brown, R., Camerini, U., Fowler, P. H., Muirhead, H., Powell, C. F., and Ritson, D. M., *Nature*, **163**, 47 (1949).

³ The existence of successive multiple nuclear disintegrations is referred to in Bridge, H. S., Hazen, W. E., Rossi, B., and Williams, R. W., *Phys. Rev.*, **74**, 1083 (1948).

⁴ Bridge, H. S., and co-workers, *loc. cit.*

⁵ Rochester, G. D., and Butler, C. C., *Proc. Roy. Soc.*, **61**, 307 (1948).

⁶ Jánossy, L., and Rochester, G. D., *Proc. Roy. Soc.*, **A**, **183**, 181 (1945). Meyer, H. A., Schwachheim, G., and Wataghin, A., *Phys. Rev.*, **74**, 846 (1948). Cocconi, G. (unpublished).

⁷ Rochester, G. D., *Proc. Roy. Soc.*, **A**, **187**, 464 (1946).

DISSOCIATION EXTRACTION

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A PROCESS has been devised for separating organic acids (or bases) of different dissociation constants from each other by solvent extraction. It is based on the fact that, in general, weak organic acids are soluble in solvents only in the form of the free undissociated acid. In essence, the system consists of a continuous counter-current extraction column in which the solvent flows in one direction (say, upwards) and water flows in the opposite direction. The mixture to be separated is fed to an intermediate point in the column. The acids partition themselves between the solvent and the water in a way which depends on the dissociation constant (*K*) and the *pH*. The concentration of an acid in the solvent (*C_S*) is related to its concentration in the water (*C_W*) by the equation

$$\frac{C_S}{C_W} = S \cdot \frac{[H^+]}{[H^+] + K} \quad (1)$$

where *S* is the partition ratio of the acid between the solvent and water. The weaker acid is preferentially present in the solvent; for example, if the partition ratios of the two acids are equal and the first acid is taken as the weaker (*K₁* < *K₂*), then

$$\frac{C_{1S}/C_{1W}}{C_{2S}/C_{2W}} = \frac{[H^+] + K_2}{[H^+] + K_1} \quad (2)$$

This equation shows that the degree of separation can be improved up to a maximum value by reducing the hydrogen ion concentration. There is, however, a practical limit to this as it entails a reduction in the concentration of acid in the solvent (equation 1); on the other hand, this can be to some extent counterbalanced by suitable choice of solvent, which should be one with a high partition ratio.

The solvent as it flows upwards is, by repeated contact, progressively enriched in the weaker acid, and correspondingly the aqueous phase is enriched in the stronger acid. The column is operated so that the total concentration of acid in each phase is constant; thus, since the weaker acid is segregated to the top of the column, the *pH* is higher there than at the bottom, and there is a continuous *pH* gradient along the length of the column. Reflux is applied at both ends of the column. At the top it is effected by adding alkali with the ingoing water, thus removing part of the acid from the outgoing solvent. At the bottom, strong mineral acid is added with the solvent in order to displace some of the organic acid from the aqueous phase to the solvent. The condition of constancy of total acid concentration in each phase follows from the fact that additions of mineral acid and alkali are made only at the ends of the column and with the feed.

This column bears a considerable resemblance to a continuous distillation column. The material to be separated consists here of two acids of different dissociation constants, in place of two liquids of different volatilities. Both processes involve the transfer of material from one phase to another, followed by separation of the phases. The solvent phase is analogous to the vapour phase in distillation, and the weaker acid which is preferentially present in the solvent phase is analogous to the more volatile component. The process of transfer from aqueous phase to solvent is paralleled by the process of vaporization. The greater the hydrogen ion concentration in the aqueous phase, the greater will become the acid concentration in the solvent phase; in the case of distillation, the higher the temperature of the liquid, the greater becomes the pressure (or concentration) in the vapour phase. Thus temperature in distillation corresponds to hydrogen ion concentration. The weaker the acid, the more readily it passes into the solvent phase at a given *pH*; that is, a weak acid with a low value for the dissociation constant corresponds to a volatile liquid of low boiling point. The ratio on the left-hand side of equation 2 has the same meaning as the volatility ratio. Application of reflux by addition of alkali with the water corresponds to the use of a dephlegmator in distillation.

The operating conditions of the column can be calculated along similar lines to those used in distillation. An equilibrium curve can be calculated, knowing the partition ratios and the dissociation constants, or it may be determined experimentally. From this, by using a construction similar to the McCabe-Thiele construction for distillation, the number of theoretical stages required to effect a given separation can be deduced.

The analogy developed here is with continuous distillation; a similar analogy may be drawn between batch distillation and the Craig¹ separation process, which likewise depends on differences in partition ratio and dissociation constant.

It is proposed to term this process 'dissociation extraction'. A full account of this work will be