It has been possible to give an indirect demonstration of such an hypothesis.

If a 1:500 solution of 'Liquoid' is mixed with equal volume (1 ml) of a solution containing lysozyme and incubated for 30 min at 37 °C a precipitate is formed in the tube. The clear supernatant gives only partial inactivation of complement when added to the guinea pig serum. The degree of inactivation of C′ is lower the higher the amount of lysozyme added to the solution of 'Liquoid'. Figure 1 shows these results.

It has also been demonstrated that methyl-lysozyme (enzymatically inactive) and protamine sulphate act as lysozyme in reactivating the haemolytic activity of the 'Liquoid' treated serum. These substances, moreover, have a combining power higher than lysozyme.

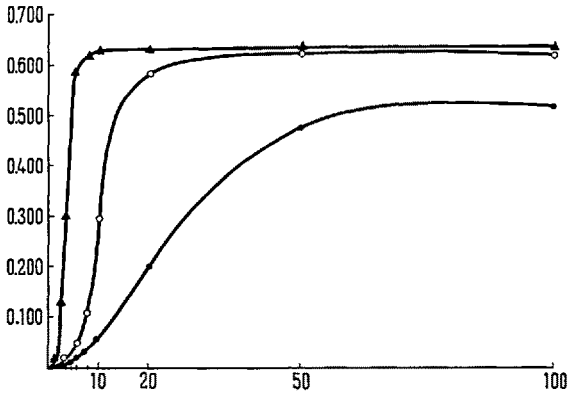

Fig. 2. Action of basic proteins to systems containing 0.2 ml EA + 0.2 ml of 'Liquoid' prepared R_3. Ordinates: values of the optical density at 5410 Å. Abscisses: μg of added proteins. •—•—• Lysozyme; o—o—o Methyl-lysozyme; Δ—Δ—Δ Protamine sulphate.

This study presented an opportunity to observe that lysozyme plays an aspecific action in its reconstituting haemolytic activity when mixed with a 'Liquoid' prepared reagent. The effect could be due to the formation of complexes with the 'Liquoid' previously fixed to the globulins of C'_3 activity furnished. Such an activity seems to be related to the basic property of lysozyme since other basic proteins act in the same way as lysozyme does.

The possibility should also be considered that a 'Liquoid' prepared R_3 fails to indicate the real content of C'_3 when used for the titration of this component in a serum containing lysozyme.

Riassunto. È stato osservato che il lisozima provoca lisi di emazie sensibilizzate in presenza di un reagente per la titolazione di C'_3, preparato con siero di cavia trattato con 'Liquoid'. È stato studiato il meccanismo di tale azione ed è stato dimostrato che essa è dovuta alla formazione di complessi tra il lisozima ed il 'Liquoid' fissato dalle globuline del siero fornite di attività di C'_3. Tale azione del lisozima è indipendente dall'attività enzimatica in quanto anche il metil-lisozima ed il solfato di protamina provocano lo stesso fenomeno.

G. Cavallo, G. Pontieri, and S. Imperato

Istituto di Patologia Generale, Università di Napoli, and Istituto di Microbiologia, Università di Pisa (Italy), July 24, 1962.

Inter-Relations of the Effects of Psilocybin on Subjective Sensation, Photopic Critical Frequency of Fusion, and Circulating Non-Esterified Fatty Acids

Psilocybin (O-Phosphoryl-4-Hydroxydimethyltryptamine), a hallucinogenic and psychotomimetic agent found in certain fungi of the genus Psilocybe has been synthesized. Its administration significantly alters biochemical, physiological, and psychological behavior. The study to be reported was designed to investigate interrelations among some of these effects.

The subjective elements of the Psilocybin syndrome include awareness of autonomic, perceptual, and general psychological changes. The autonomic effects are predominantly adrenergic; the perceptual effects commonly include distortions of color and detail and hallucination of color and form. The general psychological response includes mild intoxication and changes in psychic function that resemble those of clinical functional psychopathology. The syndrome is not that of a toxic delirium; memory and orientation are preserved.

As Psilocybin so profoundly effects vision it seemed indicated to seek a neurophysiological correlate of this effect. Takashina[1] has reported that lysergic acid diethyl amide, a drug similar in effect to Psilocybin, significantly increases photopic CFF. CFF (critical fusion of frequency) may be defined as the minimal rate at which intermittent photic stimuli are perceived as continuous illuminations. A pilot study as well as the study to be described demonstrate that Psilocybin also has such action. The existence of subjective visual changes and changes in CFF does not in itself demonstrate that these changes are related. They might reflect effects of a common process at psychological and physiological levels or they might reflect the effects of different basic processes. One method of ascertaining relations between two effects is to study their times of onset, height, and diminution. Effects that are temporally coincident are more likely to depend on a common underlying process than are changes that occur at different intervals after drug administration.

In view of the predominantly adrenergic nature of the autonomic symptomatology it seemed of interest to study, directly or indirectly, changes in circulating epinephrine and nor-epinephrine after Psilocybin administration. The direct assay of circulating epinephrine and nor-epinephrine is technically difficult and requires relatively large amounts of blood (a problem if serial determinations are necessary). It has been demonstrated, however, that circulating non-esterified or free fatty acids (FFA) vary with circulating catechol amines in a wide range of circumstances. Changes in circulating FFA are not specific to catechol amines. They depend on an intact and responsive adrenal cortex, are elevated by

[1] K. Takashina, Psychiat. Neurol. Japan *62*, 1745 (1960).

Occurrence of changes in subjective state, photopic critical frequency of fusion, and free fatty acids after administration of Psilocybin

Subject	mg/kg of Psilocybin	Initial values			Time in h after drug of definite increases [a]			Time in h after drug of marked increases [b]		
		Questionnaire	CFF	FFA	Questionnaire	CFF	FFA	Questionnaire	CFF	FFA
1	0.05	2	39	400	—	—	—	—	—	—
2	0.05	3	39	350	—	1–2*	—	—	—	—
3	0.2	3	46	410	$^1/_2$–3	$^1/_2$–4	—**	1–$2^1/_2$	1–4	—**
4	0.2	3	52	320	$^1/_2$–3	$1^1/_2$–$2^1/_2$	$2^1/_2$–4***	$^1/_2$–$2^1/_2$	$1^1/_2$–2	3–4***
5	0.2	7	36	380	1–4	$^1/_2$–$3^1/_2$	1–4	2–$2^1/_2$	1–3	$1^1/_2$–3
6	0.2	3	41	370	$^1/_2$–$2^1/_2$	1–4	1–3	1–2	2–4	$1^1/_2$–$2^1/_2$
7	0.2	4	38	400	$^1/_2$–3	$^1/_2$–4	$1^1/_2$–3	1–2	$1^1/_2$–4	2–$2^1/_2$

[a] Over 6 more positive questionnaire responses, CFF increases of over $1^1/_2$ flashes per sec, FFA increases of over 100% of initial value
[b] Over 4 more positive questionnaire responses, CFF increases of over 3 flashes per sec, FFA increases over 200%.
* ** *** Non-coincidences of effect considered in text.

thyroid and growth hormones, and are decreased by an increase in blood sugar or circulating amino acids and by hypoalbuminemia. However, at the present state of information, a marked elevation of FFA which occurs within a few minutes may be assumed to effect sympathetic stimulation or an increase in circulating catechol amines; other factors known to increase FFA require hours or days to do so[2]. HOLLISTER[3] demonstrated that Psilocybin, Lysergic acid diethylamide, and Mescaline significantly increased circulating FFA in fasting subjects. In preliminary studies we demonstrated that Psilocybin increased FFA in non-fasting subjects and that, in our experimental situation, a placebo did not so increase FFA. These studies were necessary as starvation in itself may increase catechol amine levels and anxiety in the experimental situation might also have this effect.

It was considered advisable, as it was with CFF, to study the temporal relation of changes in FFA and the subjective aspects of the Psilocybin syndrome.

Fortunately, determinations of CFF and FFA are not only meaningful but can be accomplished at regular intervals during the Psilocybin syndrome. It is similarly fortunate that a well standardized questionnaire exists that gives a quantitative estimate of subjective drug effect. ABRAMSON[4] has developed a 76-question check list that is specific for drug as opposed to placebo effect and can be accomplished at frequent intervals after drug administration.

Subjective response, CFF, FFA were determined before and at half-hourly intervals after the administration of Psilocybin for 4 h. Two subjects received 0.05 mg/kg and 5 subjects received 0.2 mg/kg of Psilocybin, the latter is the usual experimental dosage. The lesser dosage was utilized to ascertain changes in CFF and FFA at a dosage level that does not usually result in strong reactions. The subjects ate before and during the experimental procedure. The investigation of the inter-relation of effects was experimentally controlled as no one investigator had access to more than one parameter of effect. A Grass Photic Stimulator was utilized to generate the CFF stimuli, the test patch was 3 millilamberts in brightness, and intercepted a $1^1/_2$ central field; an artificial pupil was used, each value represents the average of 3 ascending runs. FFA levels were determined as described by DOLE[5].

The Table summarizes the results of these studies. Predrug subjective response, CFF, and FFA are expressed in terms of positive questionnaire responses flashes per second, and $\mu M/l$. A 'definite increase' is constituted by at least 6 more positive questionnaire responses, and increase of at least $1^1/_2$ flashes/sec in CFF, an increase of at least 100% of the pre-drug value of FFA. A 'marked increase' consists of at least 14 more positive questionnaire responses, and increase of at least 3 cycles/sec in CFF, an increase of at least 200% of the pre-drug value of FFA.

Changes in subjective response and CFF were fairly but not precisely consistent. This suggests that subjective drug effect and CFF may reflect similar basic processes. Subjective responses, however, were more consistently accompanied by CFF elevations than were CFF elevations accompanied by subjective responses. In one of the subjects who received a small dosage of Psilocybin (indicated by single asterisk) CFF increased in the absence of definite subjective response. This might be explained on the basis that photopic CFF is the more sensitive indicator, that lower dosages of Psilocybin cause autonomic but not psychotomimetic effect (autonomic activity can influence CFF), or that the experimental procedures may cause anxiety which in itself can increase CFF. The data also indicate that Psilocybin increases photopic CFF.

In one subject FFA did not increase during a marked drug response (double asterisk). In another subject FFA increased only after the other drug effects were waning or gone (triple asterisk). This suggests that a definite Psilocybin response can occur without there being a simultaneous increase in circulating epinephrine or nor-epinephrine. This in no way disputes the idea that Psilocybin effect may be mediated in whole or part through alterations in catechol amine metabolism; the suggestion is only that a simple increase in epinephrine and nor-epinephrine levels need not be involved.

Résumé. L'administration de psilocybine produit des symptômes subjectifs, augmente l'adaptation à la stimulation lumineuse intermittente de la tache jaune et augmente la quantité circulante d'acides gras estérifiés (en corrélation avec l'adrénaline et la nor-adrénaline circulantes). Les changements d'adaptation à la stimulation lumineuse intermittente sont plus constants que ceux qui se produisent dans les acides gras libres et coïncident mieux que ces derniers avec le moment d'apparition des symptômes subjectifs.

M. H. KEELER

Department of Psychiatry, University of North Carolina School of Medicine, Chapel Hill (North Carolina, U.S.A.), September 7, 1962.

[2] M. BOGDONOFF et al., Ann. int. Med. *55*, 328 (1961).
[3] L. HOLLISTER, (Abstract) Clin. Res. N.Y. *9*, 181 (1961).
[4] H. ABRAMSON, J. Psychol. *48*, 65 (1959).
[5] V. DOLE, J. clin. Invest. *35*, 150 (1956).