A Note on Properties of Pigments Produced by *Epicoccum nigrum*

The fungus *Epicoccum nigrum* when cultured under suitable conditions produces a red pigment which diffuses readily into the medium. There seems to be no agreement among investigators on the chemical nature and properties of the pigment. It has been referred to as flavipin, humic acid, caratenoids and flavinoids by various investigators[1-4]. In this paper some of the properties of the extracted crude pigment are reported.

Materials and methods. The microorganism used was *Epicoccum nigrum* (Strain 5-1-3)[5], the inoculum consisted of a suspension of spore and mycelial fragments, the medium was the Czapek Dox glucose medium. The fungus was grown as surface felt and were incubated at 25 °C exposed to 40–50 foot candles of artificial light from day light fluorescent electric tubes TL 80W/55, 5′ suspended at a suitable distance above the culture vessels. The pigment was extracted from the acidified or non-acidified medium using suitable solvents including diethyl-ether, alcohol-ether and alcohol.

Results. Solubility of the pigment. The pigment produced by *E. nigrum* was found to be soluble in water, methanol, ethanol, ether and acetone. It was sparingly soluble in butanol, chloroform and ethyl acetate. It was insoluble in butyl methyl ketone, benzene and toluene.

Chemical tests. Various tests were performed on aqueous and alcohol-ether solutions of the pigment. A yellow pigment was obtained on treatment of the pigment with excess alkaline or excess acid. The pigment gave with aqueous solutions of ferric chloride an intense blue-black colour which darkened on standing. With Brady's reagent (0.32% 2, 4, Dinitrophenyl-hydrazine in 2*N* HCl) it gave an immediate red precipitate. It reduced ammonical silver nitrate and Fehlings solution on heating.

Homogeneity of the pigment. To test for homogeneity of the crude pigment both paper and thin-layer chromatography were carried out. The solvents used was water saturated butanol-acetone – 0.5*N* HCl (12:12:1.5). Using this solvent both paper chromatography and thin-layer chromatography of an alcohol solution of the red pigment revealed the presence of red, orange and yellow fractions in varying proportions.

Absorption spectrum of the crude pigment. Alcohol solution of ether extractable pigment was used and this showed an absorption maximum in the blue violet region of the spectrum with peaks of 330–350 mU. In addition to absorption in the blue violet region, the solution showed a secondary absorption maximum in the red end of the spectrum with peaks of 1000–1010 mU.

Discussion. The findings here tend to support the presence of flavipin-like substance in the crude pigment secreted by *E. nigrum*. It is also possible that carotenoids, flavinoids and humic acid-like substance are also elaborated by the fungus. In studying the pigment produced by *E. nigrum* various investigators have followed different lines of approach and it is possible that each may be dealing with a fraction of what appears to be a complex substance. Further investigations to establish the true chemical nature and other properties of the pigment may be rewarding.

Zusammenfassung. Untersuchungen der Pigmenteigenschaften des Pilzes *Epicoccum nigrum* ergaben die Anwesenheit einer flavipinartigen Substanz (3, 4, 5-trihydroxy-6-Methylphthal-Aldehyd).

O. U. EKA

Department of Industrial Microbiology, University College, Dublin 4 (Ireland), 18 February 1970.

[1] P. C. BAMFORD, G. NORRIS and G. WARD, Trans. Br. mycol. Soc. *44*, 354 (1961).
[2] F. H. FOPPEN and O. GRIBANOVSKI-SASSU, Biochem. J. *106*, 97 (1968).
[3] J. P. MARTIN, S. J. RICHARDS and K. HAIDER, Soil Sci. Soc. Am. Proc. *31*, 657 (1967).
[4] O. U. EKAH, Ph. D. Thesis National University of Ireland (1969).
[5] B. SCHOL-SCHWARZ, Trans. Br. mycol. Soc. *42*, 149 (1959).

STUDIORUM PROGRESSUS

Comparative Pharmacopsychological Study of the Effects Produced by Psychopharmaceuticals on Verbal Interaction in a Group of Students

1. *Statement of the problem.* Pharmacopsychological research aims at establishing criteria by which the influence on human experience and behaviour of substances affecting the CNS can be reliably described in its most important dimensions. Both a review of the methods used in pharmacopsychology, such as JANKE[1] produced, and the study of newer papers in this field, reveal that the effects of psychotropic substances on the individual's social behaviour have been most inadequately investigated.

This does not apply to the clinical and therapeutic use of psychopharmaca in which target symptoms (FREYHAN[2]) such as 'affectivity', 'social adjustment', 'contact with other patients', and 'aggression' form a part of every psychiatric observation or interview. Above all, the deficiency makes itself felt in work with normal subjects, which might be expected to assess the pattern of effects (specific psychological effects) produced by the psychotropic drug under controlled conditions. The following questions must, therefore, be asked of pharmacopsychological studies: (1) Is it possible to produce a method of assessing the effects which psychotropic substances produce on the social behaviour of clinically normal individuals? (2) Can the effects of substances which are pharmacologically and clinically distinguishable be differentiated from one another and from placebos with regard to the dimensions described?

2. *Method.* We developed the following experimental design for clarifying these questions: Two clinically and pharmacologically distinguishable substances were given in random succession to student volunteers who were taking part in a psychoanalytically oriented personal ex-

perience group (BATTEGAY[3]). A modified version of BALES and SLATER's[4] interaction analysis was used to record the individuals' social behaviour within the group. Statistical evaluation included both the quantity and the affective quality of social relationship expressed in verbal activity[3,5].

2.1 *Experimental conditions.* Within the hospital setting quantification of social relationships is very diffitult and dependent on a number of environmental factors, so we carried out our study with 6 normal subjects on 6 separate days. Each session began at 08.00 h and took place at weekly intervals. The subjects came to the psychiatric out-patients department at 08.00 h, were given their medicament at 08.10 h and the group session started at 08.50 h. The 40 min waiting period was spent *together* (under the control of one of the investigators) in the experimental room.

The students were paid for their participation (SFr. 20.— per session) and committed to taking part in all the sessions from the first to the last. They included students from various faculties who, with one exception, had not known one another beforehand. In the course of a first unrecorded session (without medication) they were made acquainted with the methods to be used. The subjects formed a loose circle together with the doctor leading the group, while a psychologist sitting in the background recorded the proceedings.

2.2 *Procedure.* Before each of the 6 group sessions (days) 2 of the 6 subjects received each of the 3 preparations: Desipramine (Pertofran®), Thioridazine (Melleril®) and the Placebo. Each of the participants, apart from the group leader, received each of the preparations twice in the course of the 6 days. This procedure led to a Latin square in which each of the 3 preparations (A, B, C) appears twice in each column (session). This square, with random allocation of the medicaments, is given below Table I.

2.3. *Measuring group interaction.* For measuring group interaction we limited ourselves to the *manifest verbal content of spoken communication.* Any other procedure was not indicated in the present state of our knowledge about processes of interaction in a non-therapeutic personal experience group, especially when we did not know any of the subjects beforehand. In particular, we had to do without assessment and interpretation of non-verbal communication, although non-verbal communication can make up an important part of the psycho-dynamic process.

We used a modified version of BALES's and SLATER's[4] interaction analysis for recording the verbal happenings in which verbal interaction is regarded as a happening between sender and receiver (BORGATTA[5]) and is recorded according to Table II.

Every remark by each subject was recorded according to these 6 categories. Those which unequivocally referred both to the subject under discussion and to the speaker or the person spoken to, were recorded twice. Interactions which were chronologically related to a number of sequences were recorded the appropriate number of times. The form used for recording is shown in Figure 1.

Organizing the study on the basis of a Latin square means that we can separate any variations between members of the group, or from one day to another (effect of learning), from the total variability. Using a double blind technique for administering the medicaments prevents the group leader from influencing the results. No difficulty was found in attributing each remark to one of the 6 categories during the actual course of the group discussions. On the other hand it is clear that records made by different people could not necessarily be compared with one another, and that the subjective judgement of the recorder provides a systematic source of error in the results. No attempt was made to assess inter-scorer reliability.

3. *Results.* The data submitted to statistical assessment consist of sender analysis and receiver analysis of the verbal contact. 'Sender analysis' includes all the contacts initiated by the subject; 'receiver analysis' includes contacts directed towards the subject.

3.1. *Total of all verbal interaction.* The first questions which we wanted to answer by analysis of variance were the following: (1) Do the preparations show any difference with regard to the total amount of contacts made? (2) Does the number of contacts made show any trend during the course of a session, i.e., is there a difference between the 4 phases of a session in the course of the 6 days? (3) Does the number of contacts made show a trend over the whole experimental period, i.e., is there a difference between the 6 days? (4) Do the subjects show differences in the amount of contacts made?

The results of both analyses of variance follow:

It can be seen from these analyses of variance (Tables III and IV) that only the differences between the subjects are significant ($P < 0.01$), whereas the 3 preparations, the 4 quarter-hours of each session, the 6 sessions and the other effects (interactions) show no significant differences.

Discussion. (1) It is of theoretical importance that 2 pharmacologically and clinically distinguishable substances, such as Desipramine and Thioridazine, could not be differentiated either from one another or from the Placebo in this study. We shall discuss the reasons for this. (2) It is also remarkable that no differences in verbal activity can be demonstrated, either in the course of individual sessions or over the 6 days. The graph which is shown in Figure 2 does appear to show a slight increase in the amount of verbal contact, but this is not statistically significant. The graph also shows clearly that none of the 6 subjects showed a trend towards increasing verbal activity over the 6 sessions.

Table I

	Session 1	2	3	4	5	6
Subject 1	C	B	A	C	B	A
Subject 2	A	C	B	B	A	C
Subject 3	B	C	A	C	A	B
Subject 4	B	A	C	A	C	B
Subject 5	C	A	B	B	C	A
Subject 6	A	B	C	A	B	C

A, Desipramine (Pertofran®), antidepressant, 25 mg; B, Thioridazine (Melleril®), neuroleptic, 25 mg; C, Placebo.

[1] W. JANKE, in *Das ärztliche Gespräch* (Tropon Werke, Köln 1968), p. 5.
[2] F. A. FREYHAN, Nervenarzt *34*, 181 (1963).
[3] R. BATTEGAY, *Der Mensch in der Gruppe II*, 2. Auflage (Hans Huber, Bern/Stuttgart/Wien 1969).
[4] R. F. BALES and PH. E. SLATER, in *Family, Socialisation and Interaction Process* (Eds. T. PARSONS and R. F. BALES; The Free Press, Glencoe, Illinois 1960).
[5] E. F. BORGATTA, Gen. Psychol. Monogr. *65*, 219 (1962).

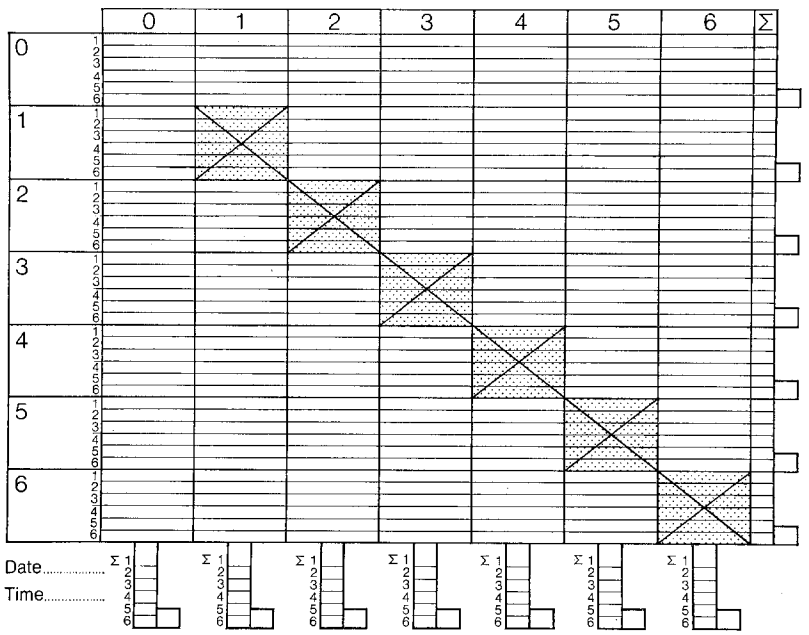

Fig. 1. Protocol sheet. Verbal interactions are protocoled at the intersection of raws (senders, including group leader) and columns (receivers, including group leader and unspecifically directed interactions).

Table II. The 6 categories of verbal interaction

(1.1) Positive emotionally tones, objective verbal remarks (lively agreement, friendly interest, confirmation, etc.).

(1.2) Affectively neutral, objective verbal remarks (unemotional interest, objective exchange of information, etc.).

(1.3) Negative emotionally toned objective verbal remarks (marked disagreement, correction, emotionally toned criticism, etc.).

(2.1) Positive emotionally toned verbal remarks referring to the self or to others (praise, expression of sympathy, agreement, etc.).

(2.2) Affectively neutral verbal remarks referring to the self or others (objective description, discussion, exchange of information, etc.).

(2.3) Negative emotionally toned remarks referring to the self or others (criticism, rejection, correction, etc. of self or others).

Table III. Analysis of senders (ANOVA for total verbal interaction)

	DF	MS	F	Significance
1. Subjects	5	13.2242	4.2406	$P<0.01$
2. Sessions	5	1.7307	0.5550	n.s.
3. Preparations	2	3.8176	1.2242	n.s.
4. Experimental error for 1–3	23	3.1185	–	–
5. Times	3	1.3089	1.0415	n.s.
6. Experimental error for 5	15	1.3568	–	–
7. Session × times	15	0.2316	0.3060	n.s.
8. Preparations × times	6	0.2579	0.3408	n.s.
9. Experimental error for 7–8	69	0.7568	–	–
Total	143			

3.2. *Influence on the 6 categories of verbal interaction.* The second step was to see whether dividing up the verbal interaction into the 6 categories described in 2.3. uncovered any differences in the effects of the 3 substances. Examination of the raw values indicated that of the 6 categories only category 1.2. (objective, emotionally neutral remarks) had a loading which made it seem worthwhile to work out the analysis of variance. However, since the loading of this category correlated with the total interactions, statistical evaluation was not promising, especially since the mean values for the 3 preparations were very close. We put together the categories 1.3. and 2.3. to form a new one, 'total of affectively negative remarks', and subjected this to analysis of variance. Since we were principally interested in the effects produced by the preparations on subjects in a setting of social relationships, we used a simplified form of the analysis of variance (Table V and VI).

Table IV. Analysis of receivers (ANOVA for total verbal interaction)

	DF	MS	F	Significance
1. Subjects	5	14.0759	4.1263	$P<0.01$
2. Sessions	5	2.0584	0.6034	n.s.
3. Preparations	2	4.5582	1.3362	n.s.
4. Experimental error for 1–3	23	3.4413	–	–
5. Times	3	1.4643	1.1060	n.s.
6. Experimental error for 5	15	1.3240	–	–
7. Sessions × times	15	0.3196	0.3767	n.s.
8. Preparations × times	6	0.7983	0.9408	n.s.
9. Experimental error for 7–8	69	0.8485	–	–
Total	143			

It is clear that differences between the psychopharmaceuticals and Placebo can, at best, be seen in the form of a tendency – under Desipramine more negative emotionally toned contacts are received – and that psychodynamically this can scarcely be interpreted. Since both analyses of variance give negative results, it can be assumed that the other categories do not differentiate between the preparations either.

4. *Discussion.* All 4 analyses of variance which have been calculated show significant differences between the subjects but none in the effects of the different preparations. Therefore, our aim to differentiate between the effects of the preparations was not achieved. The following

factors may account for this failure: (1) The latency period between administration of the medicament and beginning the experimental session (40 min) may have been too short. The medicament could not develop its full effectiveness. (2) Waiting together in the same room before beginning the actual group session may have toned down subsequent interaction. (3) The experimental period of 60 min per session may have been too short, so that differences in verbal interaction with different preparations could not be demonstrated. (4) The method of recording may have been too rough so that differences in verbal interaction were inadequately reflected. (5) The experimental method may have been unsuitable in that we adopted the analytical attitude appropriate to group therapy and did not control the topic of the discussion. Thus a topic which had been raised could be followed over a number of sessions, and only the subjects interested in the topic joined in the discussion. (6) The time of day (08.50 h) may have hindered the emotional participation of some subjects. (7) The dosage may have been too low. (8) The medicaments used may not have a sufficiently specific effect with regard to the parameters under study.

Point 4 could be improved by using tape to record the group discussion so that later checking (evaluation of the contents of verbal group interaction) would be possible. As a result of technical inadequacies in the study, points 7 and 8, which are the actual problems under study, cannot be finally answered. In future work, points 1, 2, 3 and 5 would have to be altered so that there would be a longer latency period between administration of the medicament and the beginning of the session, the waiting period could be spent apart from one another, the length of the session could be increased, and a topic suggested for each session.

Despite inadequacies in the methods used, the study is useful for showing up their limits, and for the light it throws upon the search for better ways of assessing the effects of psychopharmaceuticals on individuals in a social setting as near as possible to real-life situations.

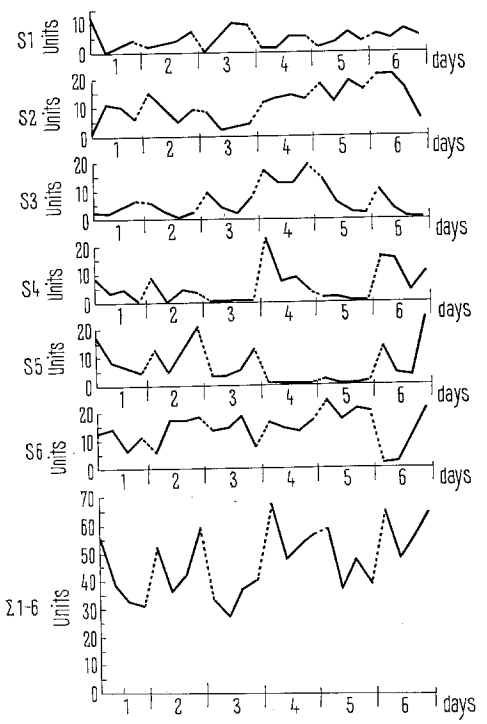

Fig. 2. Total verbal output. Graphic representation of verbal output in units of 15 min.

Table V. Analysis of senders (ANOVA for emotionally negative remarks)

	DF	MS	F	Significance
Subjects	5	1.8502	3.811	$P < 0.05$
Sessions	5	3.0616	6.306	$P < 0.001$
Preparations	2	0.4285	0.883	n.s.
Remainder	23	0.4855	–	–
Total	35			

Table VI. Analysis of receivers (ANOVA for emotionally negative remarks)

	DF	MS	F	Significance
Subjects	5	1.6678	4.338	$P < 0.01$
Sessions	5	3.1198	8.114	$P < 0.001$
Preparations	2	0.9979	2.395	$P < 0.10$
Remainder	23	0.3845	–	–
Total	35			

Zusammenfassung. In einer pharmakopsychologischen Gruppenuntersuchung wurde das Problem der Wirkung von pharmakologisch und klinisch unterschiedlichen Psychopharmaka auf die verbalen Interaktionen in einer Gruppe von gesunden Probanden angegangen. Mittelpunkt der Untersuchung war die Frage, ob Thioridazin (Melleril®), Desipramin (Pertofran®) und Placebo unterschiedlich auf Quantität und Qualität der verbalen Interaktionen der Gruppenteilnehmer wirkten. Von dieser Fragestellung wurde ein Beitrag zum Verständnis der Wirkungsweise von Psychopharmaka hinsichtlich ihrer sozialen Wirkkomponente erwartet. Der Versuch diente in erster Linie methodischen Abklärungen im Hinblick auf eine spätere Anwendung dieser Versuchsanordnung an Patientenkollektiven.

Dem Versuch lag die Struktur des Lateinischen Quadrats (6 Probanden, 6 Versuchstage, $2 \times 3 = 6$ Präparate) zugrunde. Die statistische Auswertung ergab keine Unterschiede zwischen den Präparaten untereinander und gegenüber Placebo hinsichtlich der untersuchten Parameter der verbalen Interaktionen. Hingegen fanden sich signifikante Differenzen zwischen den einzelnen Probanden in Bezug auf ihre verbale Aktivität. Die Gründe für dieses Resultat werden diskutiert. Eine Wiederholung des Versuchs an gesunden Probanden mit entsprechenden Änderungen im Procedere ist angezeigt, bevor die Versuchsanordnung bei Patientengruppen angewendet wird.

R. Battegay, R. Spiegel and K. Abt

University of Basel, Psychiatric Out-Patient Clinic and Biological and Medical Research Division, Sandoz AG., CH-4000 Basel (Switzerland), 15 December 1969.