Microbiological Assay of Indole in Cultures of *Escherichia coli* and Some Other Microorganisms

The assay of indole as a fission product of the enzyme tryptophanase causes no special difficulties in routine diagnosis of *Escherichia coli* and other microorganisms. The most common method is the assay by means of a *p*-dimethylamino-benzaldehyde reagent[1,2]; other methods are Gnezda's test employing oxalic acid[3], Salkowski's nitrose-indole reaction[4], and perhaps the test with vaniline[5]. All the reactions mentioned are, however, not specific only for indole; scatole, other indole derivatives and a number of other compounds show a similar reaction.

37 years ago, GRAY[6] was able to isolate from soil an interesting microorganism, *Pseudomonas indoloxydans*, which specifically oxidized indole to a blue staining substance: indigotine. The above microorganism was used by the authors for an assay of indole production from tryptophane in *E. coli* on a solid medium. Composition of medium: yeast extract, 3 g; *dl*-tryptophane, 5 g; disodiumphosphate, 1 g; sodium chloride, 5 g; agar, 12 g; distilled water, 1000 ml. Agar slants were made in small tubes (0.8 · 8 cm), 1–1.5 ml agar in each, and the surface was massively inoculated with the microorganism under investigation (*E. coli*). On the surface of the medium thus inoculated, a thick layer of *P. indoloxydans* (48 h culture on agar slant, cultivated at 25–28 °C) was streaked with a loop in the direction of the long axis, in the form of a line approximately 1 cm long and 0.2 cm wide. Following 24 h incubation at 25–28 °C, indole production was manifested in a more or less intense blue colouration of the site and the neighbourhood inoculated with *P. indoloxydans*. The reaction proceeds according to the following scheme:

$$\text{tryptophane} \xrightarrow[\text{\textit{E. coli} and other microorganisms}]{\text{tryptophanase}} \text{indole} \xrightarrow[\text{\textit{P. indoloxydans}}]{\text{oxidative enzyme}} \text{indigotine}$$

The production of indigotine from indole by *P. indoloxydans* is very sensitive and specific for indole. Scatole, isatine, indolacetic acid tryptophane and other indole derivatives fail to react. With *E. coli*, the reaction was absolutely reliable and agreed in all cases with the routine assay of indole by means of the chemical reaction with the *p*-dimethylaminobenzaldehyde reagent. A total of 172 microorganisms, including 109 *E. coli* strains, have so far been examined in this way. Of the other indole-positive microorganisms, only the following members of Enterobacteriaceae have hitherto been examined: *Shigella flexneri*, *Klebsiella oxytocum*, and the group Proteus-Providencia. The reaction proved to be unreliable only in the group Proteus-Providencia, probably in view of the fact that on the medium we have used, rich in tryptophane, bacteria of the Proteus-Providencia group produce a specific reddish-brown pigment[7], the production of which probably interferes with the reaction of indigotine formation by *P. indoloxydans*.

It is our opinion that the microbiological assay of indole by means of *P. indoloxydans* can be used with *E. coli* and some other microorganisms as a simple and specific diagnostic method. As has already been shown by some preexperiments, it may be possible to utilize this method for a specific and highly sensitive assay of indole, also in other biological material.

Zusammenfassung. Methode zur Indolbestimmung in Kulturen von *Escherichia coli* und manchen anderen Mikroorganismen mit Hilfe des *Pseudomonas indoloxydans*. Diese Bakterien oxydieren spezifisch Indol zum blauen Farbstoff Indigotin. Die Methode ist auch für Indolbestimmung in anderem biologischem Material optimal geeignet.

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Chlorpromazine and Fur Shaking in Mice

As a multi-response approach, the construction of ethograms has found application in psychogenetics[1] as well as in psychopharmacology[2]. In the framework of a study[3] on the relations between behavioral variables, gene action, and drug action, the effects of chlorpromazine on stereotyped behavioral components displayed by mice from four different strains were examined. The present report focuses on a specific effect of a moderate dose of the drug on behavior.

Experimental. The subjects used were 20 male mice aged $2^1/_2$ months from each of the following strains[4]: DBA/2J, C57BL/6J, B6D2F$_1$, SEC/1Gn (*se se* mice), and SEC/1Gn (*se* + mice). In each group, one half of the animals received chlorpromazine in isotonic saline (0.95 mg/kg of body weight; total volume approximately 0.17 ml), injected intraperitoneally 1 h before testing; the controls received saline. Earlier observations indicated that administering the drug in double or quadruple doses induced drowsiness and dragging with the hind legs, thus resulting in a general suppression of locomotor activity.

Frequency counts were made of 34 acts and postures exhibited by the mice when placed singly for 20 min and in pairs for 15 min in a large observation cage. Simultaneously, locomotor activity was recorded by means of

1 J. H. F. VAN ABEELEN, Genetica *34*, 79 (1963).
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3 photoelectric cells. Further details of procedure can be found elsewhere[3].

Results. The only consistent effect observed in the treated animals was a decrease in fur shaking. This component shows some resemblance to the well-known dog behavior, but in mice the shaking movement is carried out much more rapidly, and there is one single shake at a time, an act in which either the entire animal or only its front part may be involved. The frequencies for treated and untreated animals, respectively, were: DBA, 8 and 19; C57BL, 35 and 75; F_1, 30 and 43; *se se*, 15 and 29; *se* +, 9 and 41. Evaluation by means of a non-parametric combining test[5] revealed that the decrease is highly significant ($p < 0.3\%$).

The other segments of the behavioral repertoire turned out to be practically unaffected. Moreover, there was no influence at all of treatment on locomotor activity for either strain.

Discussion. Most probably, fur shaking is elicited by irritations of the skin, e.g. by salt crystals and – in the field – by ectoparasites. The finding that chlorpromazine in the dosage used tends to block specifically the release of this act, whereas other motor functions are not interfered with, may be attributed to a lowering of sensitivity to itching in drugged animals. This is in keeping with a statement made in the literature[6] that chlorpromazine reduces the ability to perceive tactile stimuli. With higher doses, this primary effect will be masked by motor impairment[7,8].

Résumé. A en juger d'après les diminutions dans l'action de se secouer le pelage, une dose moderée de chlorpromasine, n'affectant pas les autres fonctions motrices, semble reduire, chez les souris, la sensitivité aux irritations de la peau.

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The Jackson Laboratory, Bar Harbor (Maine, USA), February 15, 1966.

[5] PH. VAN ELTEREN, Bull. Inst. int. Statist. *37*, 351 (1960).

[6] J. A. SCHNEIDER and E. B. SIGG, in *Psychopharmacology* (Ed., H. H. PENNES; Hoeber and Harper, New York 1958), p. 75.

[7] This investigation was supported in part by Public Health Service Research Grant No. MH 01775, from the National Institute of Mental Health, and in part by Public Health Service General Research Support Grant No. 1 SO1 FR-05545-01, from the Division of Research Facilities and Resources.

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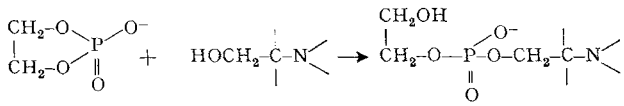
Further Observations on the Transesterification Reactions of Ethylene Phosphate with 2-Amino Alcohols

Some time ago it was reported[1] that ethylene phosphate reacts with 2-amino alcohols (ethanolamine, N,N-dimethylethanolamine, *tris*-(hydroxymethyl) amino-methane) in aqueous solution at weakly alkaline pH (8.3–9.4) to give transesterification products:



The reaction was favoured by an increase in pH and this suggested that the free NH_2 group (pKa = 9.45 in ethanolamine) played a role. This hypothesis has now been strengthened by the following experiment.

An aqueous solution (4 ml) of ethanolamine and choline chloride (3.4 *M* each, pH adjusted to 9.6 with hydrochloric acid) was made and ethylene phosphate dissolved in it (0.1 *M* calcium salt). The solution[2] was left at 37 °C for one week when it was treated in the usual way[1] with IRC-50, H^+ (6 ml) and Dowex 50, Na^+, 50–100 mesh (200 ml) resins, in order to remove the unreacted amino alcohols. In the solution obtained (recovery of phosphorus, 89%), a ratio N/P = 0.71 was found. The esterified ethanolamine and choline were determined after acid hydrolysis (HCl 1N, 1 h at 100 °C), the first by periodic acid oxidation, the second by the colorimetric procedure of HACK[3]. On a molar basis it was found that the amount of ethanolamine present corresponded to 59.5% of the total phosphorus, choline to 11.5%, the rest of the phosphorus being presumably present as glycol phosphate. The amphoteric character of the transesterification products was evidenced by passage over a column of Dowex 1 (Cl^-) at neutral pH, from which 70% of the phosphorus was recovered as expected, the retained part being again presumably glycol phosphate.

It appears therefore that the transesterification reaction is favoured by a free amino group, as partially present in ethanolamine but not in choline[4]. If this is the case, we may ask ourselves why. We have considered the following possibilities:

(1) The amino group provides nucleophilic catalysis by attacking the phosphoryl and forming a phosphoramidate intermediate. We think that this is unlikely for the following reasons: (a) We had no evidence[1] of the formation of such an intermediate in the reaction with ethanolamine. A single, amphoteric, ninhydrin positive product was obtained. (b) It appears that nitrogen bases are poor

[1] C. DEKKER and J. LECOCQ, Experientia *15*, 27 (1959).

[2] A precipitate soon appeared. In the previously reported experiments[1] on the reaction with ethanolamine alone, a similar precipitation had also been observed when the concentration of the cyclic phosphate was 0.15 *M* but not when it was 0.05 *M*. However, this difference did not affect the yields, which were the same in both cases.

[3] M. H. HACK, J. biol. Chem. *169*, 137 (1947).

[4] The precise interpretation of our result is complicated by the possibility that the transesterification products revert to the cyclic phosphate and at different rates. However, such reverse reactions, constantly tending to regenerate the cyclic phosphate should finally result in a complete hydrolysis to glycol phosphate. The relatively small amount of glycol phosphate found after one week may be taken as an indication that these reverse reactions do not take place to any great extent. A kinetic study should provide a more definite answer on this point.