INVESTIGATION OF THE FREE FORMALDEHYDE CONTENT WHEN POLIOMYELITIS VIRUS IS INACTIVATED WITH FORMALIN

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The considerable interest in the inactivation of viruses with formalin is due primarily to the preparation of antipoliomyelitis vaccine. Of the more important conditions of the inactivation process [4] which have not been considered quantitatively, there has remained the quantity of formaldehyde reacting with the virus. It is known that not all the formaldehyde added reacts with the virus, but that part of it is fixed by other components of the virus-containing fluid – amino acids, peptides and proteins – which pass into it from the synthetic medium 199 (Parker) and accumulate in the culture tissue in the process of proliferation. This bond is largely reversible.

In order to judge the quantity of formaldehyde capable of causing inactivation without essential depression of antigenicity, two values are therefore required – the total and the free formaldehyde.

Until recently, however, there has been no sufficiently reliable quantitative method of estimation of free formaldehyde [2, 8]. The only known indication was that of Taylor and Moloney [7] that, from the results of one approximate diffusion experiment, the content of free formaldehyde in monovalent poliomyelitis vaccine was about 90 %.

The method which we have devised for the determination of free formaldehyde in the presence of labile amino acid-formaldehyde and protein-formaldehyde compounds has enabled us to obtain the necessary values for the solution of this problem.

EXPERIMENTAL METHOD

We investigated different, independently prepared batches of virus fluid, obtained from a culture of kidney tissue in synthetic medium 199 and inactivated in accordance with Salk's [4, 5] recommendations by the addition of 1:4000 formalin, giving a final formaldehyde concentration of 0.1 g/l.

In each case a sample for testing was taken on the 12th day of storage of the inactivated fluid at a temperature of 37°, i.e., in the phase of completion of the reaction between formaldehyde and the components of the virus fluid. The amino-nitrogen was estimated before its partial combination with formaldehyde, in the original virus fluid without formalin. The method of estimation of free formaldehyde has been described previously by us [1], and we shall merely give here a brief account of the principle of the analysis.

The investigation was carried out in a modified Conway's dish, in which the base of the inner chamber was slightly raised so that its depth was 1.5-2.0 mm. The outer chamber was filled with the test solution. A small volume of water (0.3 ml), covering the base of the inner chamber in a thin film, absorbed the gas evolved from this solution. In a state of equilibrium (after 42 hours) a concentration of free formaldehyde was produced in the absorbing liquid in which the presence of labile formaldehyde compounds did not interfere with the subsequent estimation of the formaldehyde by a colorimetric method [6].

TABLE 1

Free Formaldehyde Content in Inactivated Virus Fluid

Serial No.	No. of virus fluid	Free formaldehyde (in g/l)	Serial No.	No. of virus fluid	Free formaldehyde (in g/l)
1	30	0,071	15	55	0,080
2	31	0,081	16	56	0,076
3	32	0,036	17	57	0,085
4	33	0,081	18	58	0,075
5	35	0,075	19	59	0,086
6	43	0,089	20	60	0,079
7	44	0,086	21	62	0,080
8	44	0,080	22	66	0,084
9	48	0,085	23	67	0,081
10	49	0,080	24	68	0,072
11	50	0,085	25	69	0,078
12	51	0,076	26	70	0,078
13	52	0,079	27	71	0,080
14	53	0,078	28	72	0,077

$\overline{\text{Mean}}$ – 0.080±0.009 g/1, δ = 0.0044, medium 199+formalin 1:4000–0.086 g/1.

TABLE 2

Total Nitrogen Content of Inactivated Virus Fluid

Serial No.	No. of virus fluid	Total nitrogen (in mg/1)
1	31	147
2	35	147
3	43	159
4	44S	147
5	44Zh	151
6	48	140
7	49	149
8	50	140
9	51	152
10	52	150
11	53	151
12	55	135
13	56	143
14	57	140
Mean		147±12 σ=6,1
Medium 199		139

Note: The figure of 156 mg/l of total nitrogen in the virus fluid before addition of solutions of formaldehyde and acetic acid corresponds by calculation to the mean total nitrogen content in the monovalent vaccines – 147 mg/l.

TABLE 3

Amino-Nitrogen Content of the Virus Fluid

Serial No.	No. of virus fluid	Amino-nitrogen (in mg/1)
1	32	126
2	33	117
3	43	124
4	44S	127
5	44Zh	121
6	60	122
7	62	115
8	65	126
9	69	122
10	70	120
11	71	120
12	72	122
13	73	121
14	75	118
Mean		121±6 σ=3,4
Medium 199		116

Note: Amino-nitrogen content of medium 199, by calculation 112 mg/l.

The total formaldehyde was estimated by the Haiberger - Nitschmann [3] method , the total nitrogen by Conway's method and the amino-nitrogen by the ninhydrin method.

EXPERIMENTAL RESULTS

It will be seen from the figures in Table 1 that the free formaldehyde content in the different batches of inactivated virus fluid was on the average 0.08 g/l. It is interesting that the medium 199 itself combined with almost tha same amount of formaldehyde, leaving 0.086 g/l uncombined .

It may be accepted that the formaldehyde in the virus fluid is combined mainly with the amino acids of medium 199, which do not undergo any essential quantitative changes in the process of cultivation of the virus (see also Tables 2 and 3).

Estimation of the total formaldehyde content of 5 batches immediately after addition of formalin showed that its value averaged 0.098 g/l (0.096-0.101 g/l), and in 20 batches at the end of the process of inactivation its value was 0.097 g/l (0.091-0.107 g/l).

The free formaldehyde thus accounted for 82.5 % of the total. The considerrable surplus of free formaldehyde reduced to a minimum the effect of small variations arising from treatment of the virus fluid, and ensured correct reproduction of its concentration.

In view of the practical importance of reproduction of the concentration of free formaldehyde , it appeared desirable to verify this conclusion by comparison with those chemical indices which reflect the concentration of those substances in the virus fluid that react with formaldehyde. The total concentration of these substances was judged by the total nitrogen present in the monovalent vaccines, and of the individual functional groups we investigated the most important — the amino group.

It may be seen from the figures in Tables 2 and 3 that the concentrations of the substances fixing formaldehyde were equally stable as the free formaldehyde. Hence it follows, in particular, that the practical recommendations directed towards the compensation of the supposed instability of the free formaldehyde concentration, and the resulting absence of standardization of the process of inactivation, for example the addition of glycine [2, 8] or further addition of formalin, have no sound basis.

We were thus able to establish that during the inactivation of the virus of poliomyelitis with formalin in accordance with the accepted recommendations [4,5] for preparation of the vaccine, the free formaldehyde content in the inactivated virus fluid had an average value of 0.08 g/l, and this concentration was readily reproduced in practice.

SUMMARY

The quantitative diffusion method of determihation of free formaldehyde has established that, inactivating poliomyelitis virus by Salk's method, the average free formaldehyde level at the end of the process was 0.08 g/l.

Formaldehyde in the virus fluid is combined mainly with amino acids passing into the fluid from the 199 medium. The free formaldehyde concentration is an easily controlled condition of inactivation .

This is confirmed by the stability of the chemical indices, the level of total nitrogen and amino nitrogen, reflecting the level of the substances interacting with formaldehyde in the virus fluid.

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