

**Table II**—Dihydrostreptomycin Recovery Data Using Fluorometric and Microbiological Methods

Product	Claim, mg./30 ml.	Found, Fluorometric Assay	Found, Microbiological Method
A	300	310	307
B	300	311	309
C	300	340	338
D	300	305	310
E	500	525	526
F	500	550	563
G	500	524	521
H	500	541	538

standard solutions containing 10, 17.15, and 24.50 mcg./ml. of dihydrostreptomycin sulfate in 0.1 N HCl.

This assay as well as the above-mentioned one regarding the guanidino groups may be applied to dihydrostreptomycin as well as to streptomycin. It does not differentiate between the biologically active product and the degradation product. Although the assay is not specific, it may be carried out in such laboratories where there are no facilities for bacteriological assay and where the potency of the dihydrostreptomycin sulfate used is known. It might also be useful for a rapid control of the suspensions during their production. Apart from these considerations the method has the advantage of being adaptable for routine assay work, whereas the biological assay for this preparation is lengthy and may yield inaccurate results in inexperienced hands.

The method besides, it not restricted to the products examined, but might be used in modified pharmaceutical preparations of a similar nature while a microbiological method is not usually the best procedure to follow in all circumstances. A number of cations and anions change the reactivity of dihydrostreptomycin and strepto-

mycin in a microbiological test. The confidence in this assay is based upon agreement between results found chemically and biologically. The results of this fluorometric assay applied to fresh commercial products compared to those obtained by microbiological method (11) using *B. subtilis* as test organism, are shown in Table II. Finally the fluorescence was found to be linearly proportional to the concentrations of dihydrostreptomycin sulfate between 5 and 12 mcg./ml. The standard deviation of standard was  $\pm 1.0\%$ .

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# Oximino Ethers: Dialkylaminoalkyl Derivatives

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**Abstract** □ Dialkylaminoalkylether derivatives of a variety of aromatic, heterocyclic, and steroidal ketone oximes were prepared and examined for their antiandrogenic effects. The compounds were prepared by two general methods. The diethylaminoethyl ether of 2-benzoylpyridine oxime exhibits interesting antiandrogenic activity in rats but the effective dose is very close to the toxic dose. In the course of this investigation other pharmacological properties of the compounds were noted.

**Keyphrases** □ Oximino ethers—dialkylaminoalkyl derivatives □ Dialkylaminoalkyl derivatives, oximino ethers—synthesis □ Antiandrogenic activity—oximino ether derivatives □ Pharmacological screening—oximino ether derivatives

In the routine screening of compounds for their endocrine effects, it was noted that compounds containing the grouping  $=N-O-(CH_2)_n-N-(R)_2$  lowered the weights of the sex organs of male rats, indicating a pos-

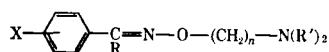
sible antiandrogenic effect.<sup>1</sup> To explore this interesting lead, a series of dialkylaminoalkyl oximino ethers was prepared from a variety of aromatic, heterocyclic, and steroidal ketones and their effects on the preputial glands, seminal vesicles, and prostate gland were examined. Other biological parameters of the oximino ethers were examined as described below.

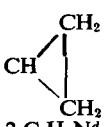
The compounds listed in Tables I and II were prepared by two general methods as shown in Scheme I.<sup>2</sup> Method A was used in those cases where the ketone oxime was readily available (1-4) and did not contain a reactive functional group. In case of steroidal ketones containing the reactive hydroxyl group, Method B was employed. The *O*-alkylated hydroxylamine derivative

<sup>1</sup> The biological data herein reported was obtained by Drs. S. Tolksdorf, R. Neri, and R. Taber of the Biological Division of the Schering Corp.

<sup>2</sup> After this work was completed, similar procedures have recently been described. See Reference 1.

Table I—Compounds of Formula

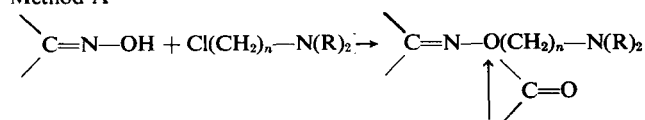


Compd.	X	R	n	R'	Method	Yield	B.p., °C.	mm.	Formula	Analysis, %	
										Calcd.	Found
I	H	C <sub>6</sub> H <sub>5</sub> <sup>a</sup>	2	CH <sub>3</sub>	A	73	185–187	1	C <sub>17</sub> H <sub>20</sub> N <sub>2</sub> O	C, 76.08 H, 7.51	C, 76.26 H, 7.75
II	H	<i>o</i> -NH <sub>2</sub> , C <sub>6</sub> H <sub>4</sub>	2	C <sub>2</sub> H <sub>5</sub>	A	52	184–190	3	C <sub>19</sub> H <sub>25</sub> N <sub>3</sub> O	N, 10.44 C, 73.28 H, 8.09	N, 10.19 C, 73.39 H, 7.53
III	<i>p</i> -OH	<i>p</i> -CH <sub>3</sub> , C <sub>6</sub> H <sub>4</sub>	2	CH <sub>3</sub>	B	42	<sup>b</sup>		C <sub>18</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub>	N, 13.49 C, 72.25 H, 7.43	N, 13.07 C, 71.84 H, 7.14
IV	<i>p</i> -CH <sub>3</sub>	<i>p</i> -OCH <sub>2</sub> CH <sub>2</sub> -N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> , C <sub>6</sub> H <sub>4</sub>	2	CH <sub>3</sub>	B	51	223–227	0.8	C <sub>24</sub> H <sub>35</sub> N <sub>3</sub> O <sub>2</sub>	N, 9.39 C, 72.51 H, 8.05	N, 9.28 C, 72.81 H, 8.61
V	<i>p</i> -OCH <sub>3</sub>	2-C <sub>4</sub> H <sub>9</sub> S <sup>c</sup>	2	CH <sub>3</sub>	B	40	196–198	1.2	C <sub>16</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub> S	N, 10.57 C, 63.14 H, 6.62	N, 10.53 C, 63.16 H, 6.76
										N, 9.21	N, 8.94
VI	<i>p</i> -OCH <sub>3</sub>		2	CH <sub>3</sub>	B	70	148–152	4	C <sub>16</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub>	C, 68.67 H, 8.45	C, 68.73 H, 8.36
VII	H	2-C <sub>6</sub> H <sub>4</sub> N <sup>d</sup>	2	CH <sub>3</sub>	A	69	166–169	3	C <sub>16</sub> H <sub>19</sub> N <sub>3</sub> O	N, 10.68 C, 71.34 H, 7.11	N, 10.75 C, 71.58 H, 6.96
VIII	H	2-C <sub>6</sub> H <sub>4</sub> N	2	C <sub>2</sub> H <sub>5</sub>	A	47	180–185	1.5	C <sub>18</sub> H <sub>23</sub> N <sub>3</sub> O	N, 15.60 C, 72.69 H, 7.80	N, 15.90 C, 72.71 H, 7.40
IX	H	<i>N</i> -CH <sub>3</sub> -4-C <sub>6</sub> H <sub>9</sub> N <sup>e</sup>	2	CH <sub>3</sub>	A	27	150–154	1	C <sub>17</sub> H <sub>27</sub> N <sub>3</sub> O	N, 14.13 C, 70.55 H, 9.40	N, 13.97 C, 70.46 H, 9.50
X	<i>p</i> -OCH <sub>3</sub>	<i>p</i> -OCH <sub>3</sub> , C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	2	CH <sub>3</sub>	B	25	223–227	2	C <sub>20</sub> H <sub>26</sub> N <sub>2</sub> O <sub>3</sub>	N, 14.52 C, 70.15 H, 7.65	N, 14.29 C, 70.40 H, 7.38
XI	-CCH <sub>3</sub>	<i>p</i> -CCH <sub>3</sub> , C <sub>6</sub> H <sub>4</sub> CH(C <sub>2</sub> H <sub>5</sub> )	2	C <sub>2</sub> H <sub>5</sub>	A	45	228–230	1	C <sub>24</sub> H <sub>34</sub> N <sub>2</sub> O <sub>3</sub>	N, 8.18 C, 72.33 H, 8.60	N, 7.80 C, 72.51 H, 8.32
XII	<i>p</i> -OCH <sub>3</sub>	<i>p</i> -OCH <sub>3</sub> , C <sub>6</sub> H <sub>4</sub> CH(C <sub>2</sub> H <sub>5</sub> )	2	CH <sub>3</sub>	B	29	219–220	1	C <sub>22</sub> H <sub>30</sub> N <sub>2</sub> O <sub>3</sub>	N, 7.03 C, 71.32 H, 8.16	N, 7.14 C, 70.98 H, 8.45
XIII	<i>p</i> -OCH <sub>3</sub>	<i>p</i> -OCH <sub>3</sub> , C <sub>6</sub> H <sub>4</sub> CH(C <sub>2</sub> H <sub>5</sub> )	3	CH <sub>3</sub>	A	50	222–224	1	C <sub>23</sub> H <sub>32</sub> N <sub>2</sub> O <sub>3</sub>	N, 7.56 C, 71.84 H, 8.39	N, 7.90 C, 72.20 H, 8.10
XIV	H	2-C <sub>6</sub> H <sub>4</sub> N-CH <sub>2</sub> <sup>f</sup>	2	CH <sub>3</sub>	A	72	178–182	3	C <sub>17</sub> H <sub>21</sub> N <sub>3</sub> O	N, 7.29 C, 72.05 H, 7.47	N, 7.22 C, 72.34 H, 7.34
										N, 14.83	N, 14.71

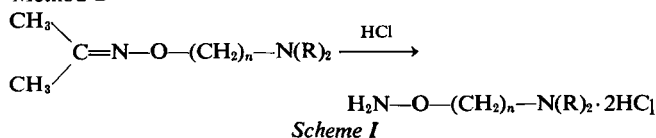
<sup>a</sup> This compound was isolated and characterized as the picrate derivative in Reference 1. <sup>b</sup> M.p. 137–138°, recrystallized from benzene. <sup>c</sup> 2-Thienyl.  
<sup>d</sup> 2-Pyridyl. <sup>e</sup> 1-Methyl-4-piperidyl. <sup>f</sup> 2-Picolyl.

(5, 6) required for Method B was prepared by the hydrochloric acid hydrolysis of the dialkylaminoalkyl ether derivative of acetone oxime, prepared by Method A from the readily available acetone oxime. The substituted hydroxylamine was isolated as the dihydrochloride. The reaction of the latter compound with a ketone was carried out in a pyridine–ethanol solution to give the monohydrochloride of the desired oxime ether, from which the free oxime ether was obtained by neutralization with sodium bicarbonate.

## Method A



## Method B



In many cases, the oxime ethers were high-boiling viscous oils from which crystalline hydrochlorides could not be obtained. This is probably due to a mixture of syn- and anti-compounds and no efforts were made to separate these mixtures.

In addition, the diethylcarbamyl oxime esters were prepared from 2-benzoylpyridine oxime and androsterone oxime by reaction of the latter compounds with diethylcarbamylchloride. These compounds were devoid of any biological activity.

Compound VIII, Table I, was the most active anti-androgenic compound in this series. When administered to immature male rats daily at 100 mg./kg. orally for 6 weeks, the preputial, seminal vesicle, and prostate weights were reduced to 65, 76, and 73% of controls, respectively. No major alterations in the histological sections of these organs were noted and the compounds did not interfere with spermatogenesis. However, the minimal effective dose was very close to the toxic dose of this compound (LD<sub>50</sub>, 108–143 mg./kg.).

Compound XVII, Table II, showed very potent anti-

Table II—Compounds of Formula  $\text{C}=\text{N}-\text{O}-(\text{CH}_2)_n-\text{N}(\text{R})_2$ 

Compd.	Ketone	R	Method	Yield, %	B.p., °C.	mm.	Formula	Analysis, %		M.p., °C.	Formula	Analysis, %	
								Calcd.	Found			Calcd.	Found
XV	Indanone-1	CH <sub>3</sub>	A	61	155–157	5	C <sub>13</sub> H <sub>18</sub> N <sub>2</sub> O	C, 71.52 H, 8.31 N, 12.83	C, 71.32 H, 7.37 N, 12.73	174–175	C <sub>13</sub> H <sub>18</sub> N <sub>2</sub> O·HCl	C, 61.28 H, 7.52	C, 61.35 H, 7.86
XVI	Fluorenone	CH <sub>3</sub>	A	69	176–180	1	C <sub>17</sub> H <sub>16</sub> N <sub>2</sub> O	C, 76.66 H, 6.81 N, 10.52	C, 76.69 H, 6.86 N, 10.21	193–195	C <sub>17</sub> H <sub>16</sub> N <sub>2</sub> O·HCl	C, 67.43 H, 6.33	C, 67.78 H, 6.66
XVII	5- <i>H</i> -Dibenzo[ <i>a,d</i> ]cycloheptene-5-one	CH <sub>3</sub>	A	80	205–210	2	C <sub>19</sub> H <sub>18</sub> N <sub>2</sub> O	C, 78.05 H, 6.90 N, 9.58	C, 78.40 H, 6.66 N, 8.91	232–235	C <sub>19</sub> H <sub>18</sub> N <sub>2</sub> O·HCl	C, 69.36 H, 6.43	C, 69.21 H, 6.26
XVIII	7-Chloro-5 <i>H</i> -dibenzo[ <i>a,d</i> ]cycloheptene-5-one	CH <sub>3</sub>	A	42 <sup>a</sup>						268–270	C <sub>19</sub> H <sub>17</sub> ClN <sub>2</sub> O·HCl	C, 62.74 H, 5.50 Cl, 5.32	C, 62.44 H, 5.83 Cl, 5.19
XIX	Isatin	CH <sub>3</sub>	B	58		<sup>b</sup>	C <sub>12</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>	C, 61.78 H, 6.48 N, 18.02	C, 62.17 H, 6.59 N, 17.86	205–207	C <sub>12</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub> ·HCl	C, 53.42 H, 5.96	C, 53.19 H, 6.09
XX	Estrone	CH <sub>3</sub>	B	68		<sup>c</sup>	C <sub>27</sub> H <sub>32</sub> N <sub>2</sub> O <sub>2</sub>	C, 74.12 H, 9.05 N, 7.86	C, 73.87 H, 8.95 N, 7.15	243–245	C <sub>27</sub> H <sub>32</sub> N <sub>2</sub> O <sub>2</sub> ·HCl	C, 67.23 H, 8.46	C, 67.65 H, 8.13
XXI	Estrone	C <sub>6</sub> H <sub>5</sub>	B			<sup>a</sup>				222–223	C <sub>27</sub> H <sub>34</sub> N <sub>2</sub> O <sub>2</sub> ·HCl	C, 68.30 H, 8.84	C, 67.89 H, 8.69
XXII	epi-Androsterone	CH <sub>3</sub>	B			<sup>a</sup>				242–243	C <sub>23</sub> H <sub>36</sub> N <sub>2</sub> O <sub>2</sub> ·HCl	C, 66.23 H, 9.91	C, 65.90 H, 9.59
XXIII	Dehydro-epi-androsterone	CH <sub>3</sub>	B	52		<sup>d</sup>	C <sub>23</sub> H <sub>34</sub> N <sub>2</sub> O <sub>2</sub>	C, 74.57 H, 9.91 N, 7.25	C, 74.59 H, 10.11 N, 6.86	249–251	C <sub>23</sub> H <sub>34</sub> N <sub>2</sub> O <sub>2</sub> ·HCl	C, 67.20 H, 9.59	C, 66.48 H, 9.59 <sup>e</sup>
XXIV	Androsterone	CH <sub>3</sub>	B	85		<sup>f</sup>	C <sub>23</sub> H <sub>36</sub> N <sub>2</sub> O <sub>2</sub>	C, 73.35 H, 10.71 N, 7.44	C, 73.60 H, 10.63 N, 7.03	247–248	C <sub>23</sub> H <sub>36</sub> N <sub>2</sub> O <sub>2</sub> ·HCl	C, 66.23 H, 9.91	C, 66.69 H, 10.05
XXV	Testosterone	CH <sub>3</sub>	B	31		<sup>a</sup>				224–225	C <sub>28</sub> H <sub>38</sub> N <sub>2</sub> O <sub>2</sub> ·HCl·H <sub>2</sub> O	C, 64.38 H, 9.61	C, 64.26 H, 9.54
XXVI	Griseofulvin	CH <sub>3</sub>	B			<sup>g</sup>	C <sub>21</sub> H <sub>27</sub> ClN <sub>2</sub> O <sub>4</sub>	C, 57.46 H, 6.20 N, 6.38	C, 57.70 H, 6.35 N, 6.51				

<sup>a</sup> Isolated as hydrochloride salt. <sup>b</sup> M.p. 124–126° recrystallized ethyl acetate. <sup>c</sup> M.p. 160–162° from methylene chloride-pentane. <sup>d</sup> M.p. 119–120° from hexane. <sup>e</sup> Calcd.: N, 6.82. Found: N, 6.59. <sup>f</sup> M.p. 110–111° from hexane. <sup>g</sup> M.p. 136–138° from isopropyl ether after preliminary chromatography on alumina using ethyl acetate as eluent.

depressant properties in the cat at an oral dose of 0.25 mg./kg. Severe side effects, whining, ataxia, and mydriasis limit the usefulness of this compound.

Compound XVI, Table II, showed potent anticonvulsant activity (maximum electroshock) in mice at an intraperitoneal dose of 3 mg./kg.; however, the compound causes severe diarrhea at this dose.

Compound XX, Table II, lowers the serum cholesterol levels of male rats at an oral dose of 30 mg./kg. but the compound is estrogenic at this dose.

### EXPERIMENTAL<sup>3</sup>

**Oximes**—The oximes required for Method A are known compounds but a standard synthetic procedure was used as follows: a mixture of ketone (0.2 mole), hydroxylamine hydrochloride (0.2 mole + 25% excess), 100 ml. of pyridine, and 200 ml. of ethanol was heated on a steam bath for 4–6 hr. The excess solvents were removed *in vacuo* on the steam bath and the residue was poured into a large volume of water (about 500 ml.), allowed to crystallize, and the crude product was recrystallized from dilute ethanol.

**Oxime Ethers**—*Method A*—In a typical example, 2.5 g. (0.11 mole) of sodium was dissolved in absolute ethanol (200 ml.) and 0.1 mole of the oxime was added. The mixture was heated under reflux for 30 min. and 0.1 mole of freshly distilled dialkylaminoalkyl chloride was added dropwise. The heating and stirring was continued for an additional 4 hr., the excess ethanol was distilled *in vacuo*, and the residue was added to water and extracted with ether. The ether solutions were extracted with dilute (10%) hydrochloric acid, the aqueous acid solution was basified with ammonium hydroxide, extracted with chloroform, washed with water, and the solvent distilled. The residue was processed as indicated in Tables I and II.

**Dimethylaminoethoxy Acetone Oxime**—A mixture of 73 g. (1.0 mole) of acetoneoxime, 157 g. of dimethylaminoethylchloride hydrochloride, 427 g. of potassium carbonate, and 1.5 l. of benzene was heated under reflux with stirring for 15 hr. After cooling, the inorganic salts were filtered off and the filtrate was concentrated to an oil which was distilled, b.p. 85–90°/50 mm.; yield 96.8 g. (67%).

*Anal.*—Calcd. for C<sub>7</sub>H<sub>16</sub>N<sub>2</sub>O: C, 58.30; H, 11.18. Found: C, 58.63; H, 11.30.

Similarly, diethylaminoethoxy acetone oxime was prepared, b.p. 109–112°/45 mm.; yield 57%.

*Anal.*—Calcd. for C<sub>9</sub>H<sub>20</sub>N<sub>2</sub>O: C, 62.74; H, 11.70. Found: C, 62.57; H, 11.46.

**Dimethylaminoethoxyamine Hydrochloride**—Twenty-eight grams of the above-substituted oxime was heated under reflux with 170 ml. of 10% hydrochloric acid for 16 hr. The solution was concentrated to dryness *in vacuo* and the residue was washed with dry ether. The residue was dissolved in methanol and the product was precipitated with ether, m.p. 180–182°; yield 21 g. (61%).

*Anal.*—Calcd. for C<sub>4</sub>H<sub>12</sub>N<sub>2</sub>O·2HCl: C, 27.56; H, 7.95; N, 15.82. Found: C, 27.17; H, 8.32; N, 15.96.

Neutralization of the dihydrochloride gave the free hydroxylamine, b.p. 108–109° (170 mm.), *n*<sub>D</sub><sup>25</sup> 1.4390.

*Anal.*—Calcd. for C<sub>4</sub>H<sub>12</sub>N<sub>2</sub>O: C, 46.13; H, 11.61; N, 26.90. Found: C, 46.12; H, 11.53; N, 27.15.

Using the same procedure, diethylaminoethoxyamine dihydrochloride was obtained in 66% yield, m.p. 115–118°.

*Anal.*—Calcd. for C<sub>6</sub>H<sub>16</sub>N<sub>2</sub>O·2HCl: C, 35.29; H, 8.82. Found: C, 35.22; H, 9.19.

**Dimethylaminoethyl Ether of Estrone Oxime**—*Method B*—A mixture of 2.7 g. of estrone, 2.0 g. of dimethylaminoethoxyamine dihydrochloride, 10 ml. of pyridine, and 20 ml. of ethanol was refluxed for 3 hr. on the steam bath. The excess solvent was removed *in vacuo* and the residue was suspended in water and neutralized with sodium bicarbonate. The product was filtered, washed thoroughly with water, and recrystallized from the indicated solvent after air drying.

**Diethylcarbamy Derivatives**—A mixture of 7.9 g. (0.04 mole) of 2-benzoylpyridine oxime, 4.7 g. of diethylcarbamy chloride, 13.8 g. of potassium carbonate, and 150 ml. of toluene was heated under reflux with stirring for 6 hr. After cooling, the mixture was filtered and the toluene solution was extracted with dilute hydrochloric acid, the acid solution was neutralized with ammonium hydroxide, and extracted with chloroform. The chloroform solution was washed with water, the solvent was removed, and the residue was triturated with hexane. The product was recrystallized from hexane, yield 6.5 g., m.p. 55–56°.

*Anal.*—Calcd. for C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub>: C, 68.66; H, 6.44; N, 14.13. Found: C, 68.53; H, 6.50; N, 14.23.

Similarly, androsterone oxime was converted to the diethylcarbamy ester, m.p. 185–186°, from benzene-petroleum ether.

*Anal.*—Calcd. for C<sub>24</sub>H<sub>40</sub>N<sub>2</sub>O<sub>3</sub>: C, 71.24; H, 9.97. Found: C, 71.27; H, 10.06.

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<sup>3</sup> Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by Mr. Edwin Conner of these laboratories.

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# Application of Lowry Protein Determination to Influenza Vaccine

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**Abstract** □ The Lowry protein test has been successfully applied to the determination of microgram amounts of protein in influenza vaccine. Because of the tendency of the virus to aggregate, additional techniques such as sonication were needed. Precision among replicates was good; the greatest difference of a single value from the average was 1.7%.

**Keyphrases** □ Influenza vaccine—protein determination □ Lowry protein test—analytic method □ Folin reagent—color formation □ Colorimetric analysis—spectrophotometer

The precise determination of protein concentration was considered essential as part of the control measures applied to influenza vaccine.<sup>1</sup> The Lowry (1) modification of the Folin-Ciocalteu (2) test was selected as being one of the most sensitive methods.

Experience has indicated that the Lowry test should not be applied to a new protein with its environment without research and/or development pertaining to the new application. In general, the procedure involved precipitation of protein from the vaccine sample with trichloroacetic acid, centrifugation, and application of the Lowry test to the pellet.

## METHODS AND MATERIALS

Vaccine used in this work contained 0.85% w/v sodium chloride and 0.2% w/v gelatin. The same lot of concentrated vaccine was used for all determinations except the study of precipitation at different concentrations of trichloroacetic acid.

The standard protein for the Lowry test was crystallized bovine plasma albumin.<sup>2</sup> For each series of test samples a solution was made up of 10 mg. in 200 ml. aqueous 0.85% sodium chloride. This solution is stable at 6° for at least 1 month and is used for the Lowry test without previous precipitation.

The Lowry reagents are those originally described in the literature and are analytical reagent grade. They are combined to give Solutions A, B, and C according to the method of Oyama and Eagle (4).

The Folin reagent<sup>3</sup> is diluted 1 part with 1 part deionized or

distilled water just before use. The original concentrate should be stored at about 6°.

Trichloroacetic acid (TCA), 30% w/v, is kept as a stock solution in a dark bottle at about 6°. This solution is stable for at least 1 month under these conditions. As needed for treatment of the test samples, a 10% w/v and a 5% w/v solution were made up from the stock solution.

Before pipeting out test samples, it is important to sonicate<sup>4</sup> the entire vaccine solution for a minimum of 1 min.

For each test, 2 ml. of sample solution was pipeted into a 13-ml. conical centrifuge tube having a flathead glass stopper. To each tube is added 2 ml. 10% w/v TCA plus 1 ml. 5% w/v TCA. The 5-ml. volume is used to give good dissolution of gelatin while still keeping the volume small enough to allow mixing in the centrifuge tube. These tests should be carried out in triplicate. The suspension briefly is mixed with a mixer.<sup>5</sup> The suspension is centrifuged<sup>6</sup> for 30 min. at 3,000 r.p.m. using a bucket-type rotor of 6-in. radius. The supernatant is discarded. TCA, 5 ml. of 5%, is added, and the pellet is dispersed by brief mixing with the mixer. After a second centrifugation and decantation, the drop of remaining supernatant is removed from the lip of the tube with absorbent tissue.

The Lowry test is performed on the precipitate. Using a total volume of 10 ml. of Reagent C for each tube, 5 ml. of the reagent was pipeted into the tube and sonicated to dissolve. The extract was decanted into a separate test tube and the extraction was repeated with a second 5 ml. of the reagent. The extracts were combined and mixed with the mixer.

Depending upon the expected concentration of protein, as indicated by experience, aliquots of the extract are taken for completion of the Lowry test. In the author's experience the volume has ranged from 1 to 2.5 ml. When needed, fresh Reagent C is added to a total volume of 2.5 ml. A dilution correction is used later. One milliliter of deionized or distilled water is now added and mixed with the mixer. For the vaccine protein tests, the remainder of the Lowry test is continued as for the protein standards and reagent blanks after Reagent C has been added to them.

For the protein standards, 1 ml. of the stock albumin solution (50 mcg. albumin) is placed in each of three tubes.

For reagent blanks, 1 ml. of deionized water is placed in each of two tubes. (Only one is needed; the second is a precautionary measure.)

To the standards and blanks 2.5 ml. Reagent C is added and mixed with the mixer.

The following applies to all solutions. Let stand in Reagent C for 10 min. or slightly longer. Jet in 0.25 ml. of diluted Folin reagent

<sup>1</sup> As produced at Eli Lilly and Co., Indianapolis, Ind.

<sup>2</sup> Armour Pharmaceutical Co., Chicago, Ill.

<sup>3</sup> Hartman-Leddon Co., Philadelphia, Pa., and Fisher Scientific Co., Chicago, Ill.

<sup>4</sup> Sonogen Automatic Cleaner, Branson Instruments, Inc., Stamford, Conn.

<sup>5</sup> Vortex-Genie, Scientific Industries, Inc., Springfield, Mass.

<sup>6</sup> Size 2, model V, International Equipment Co., Boston, Mass.