group, fractured and growth hormone treated, the rise occurred but was less than that in the fractured untreated group.

The mean level of nitrogen excretion for each of the 4 groups during the control period was essentially the same, running from 142 mg to 144 mg of nitrogen per day with a standard deviation of from ± 3.5 to ± 5.1. In the 5-day postoperative period the average daily excretion of the control group rose to 165 ± 4.8† while that of the control group given hormone rose only to 145 ± 4.3. This represents a daily retention of 20 mg of nitrogen for the treated group as compared with the controls for the same period. The average daily excretion of the fractured group rose from 142 ± 5.1 to 223 ± 9.0, while that of the group fractured and given growth hormone rose only from 143 ± 3.5 to 199 ± 8.0, representing a retention over the untreated fractured group of 24 mg of nitrogen per day. The differences between the groups noted in this first 5-day postoperative period were all significant having p values of .05 and less.[7]

In the 5-day period after growth hormone was stopped, the nitrogen excretion of the control group averaged 30 mg per day higher than in the previous period, while that of the growth hormone treated group rose 58 mg. Also, the nitrogen excretion of the group fractured and given growth hormone was higher on the average than that of the fractured untreated group.

Discussion. In these experiments the retention of nitrogen following growth hormone was approximately the same in the fractured and unfractured rats when they were compared with their respective untreated controls. It would appear that the effect of growth hormone was to lower the base line of nitrogen excretion upon which the stress of fracture was superimposed. Cuthbertson, Webster and Young[5] reported that a crude extract of the anterior pituitary completely prevented the rise of nitrogen excretion after fracture, although it did not promote nitrogen storage beyond that occurring in the uninjected control period. There are several important differences between our experiments and theirs which could explain the different results obtained. In the present experiment 2 legs were fractured, creating a greater tendency to breakdown of protein than in the experiment of Cuthbertson, Webster and Young, where only one leg was broken. In addition younger animals were used in our experiments, which may have reduced the nitrogen retaining effect of the growth hormone.

Summary. The daily urinary nitrogen excretion was followed in growth hormone treated rats with bilateral femur fractures. The normal and fractured rats given growth hormone showed approximately the same decrease in nitrogen excretion when compared with their respective controls.

† Standard deviation of the mean.

[7] Fisher, R. A., *Statistical Methods for Research Workers*, Oliver and Boyd, London, 1936.

15367

Measured Dose of Gamma Hexachlorocyclohexane (γ 666) Required to Kill Flies and Cockroaches, and a Comparison with DDT.

J. SAVIT, J. J. KOLLROS, AND J. M. TOBIAS. (Introduced by R. W. Gerard).

From the University of Chicago Toxicity Laboratory and Department of Physiology.*

Hexachlorocyclohexane† or "666" has been said to be more toxic than DDT‡ for certain insects[2,5,7,8,9,11] and mammals.[3] As was also true of DDT until recently,[13] however, most of the information is in terms of percent kill after exposure for a given time to a given environmental concentration of the

* This work was carried out under contract with the Medical Division of the Chemical Warfare Service.

agent. There are very few data available[4] on the actual measured dose (mg per kg) required to kill insects. Exposure data are invaluable from the point of view of field application,[6] but for studies on mechanism of action, it is essential to know the approximate dose which an insect must receive to produce death or some other specific physiological effect.[14,15] Therefore, measurements have been made of the LD_{50} (mg per kg required to kill 50%, determined graphically and by the method of Bliss[1]) of the active gamma isomer of hexachlorocyclohexane for flies *(Musca domestica* and *Calliphora spp.)* and the cockroach *(Periplaneta americana).*

Methods. Since the gamma isomer of hexachlorocyclohexane is far more toxic than the alpha, beta or delta forms, and is the important constituent of manufactured 666, it has been used in pure form (m.p. 112.5°C)[12] throughout, in these studies. All roaches have been unanesthetized, but flies were lightly anesthetized with ether during the administration of the toxic agent.

Methods of administration have been essentially the same as those used in a similar study of DDT.[13] The toxic agent, dissolved in acetone, was delivered through a 27-gauge hypodermic needle, from a 0.25 cc tuberculin syringe fitted with a micrometer screw-driven piston.[10] Such an instrument is capable of delivering volumes of liquid of the order of 0.2 mm^3 with about 5% accuracy. For injection purposes, in the roach, the needle was made to enter the abdomen beneath a sternite and near its hinge-like point of attachment. Upon withdrawal of a needle so inserted the sternite falls back into place and seals the hole. No leakage has been seen following such a procedure. For surface application, roaches were held by the wings. The γ 666 was then deposited beneath the wings on the dorsum of the thorax from the instrument described above, but now through a blunted needle held flat against the body surface. When folded back into place the seldom-moved wings protect against loss of dry powder remaining after solvent evaporation. Control animals were given acetone without the γ 666 (Tables I and II).

Roaches were kept in large battery jars, in groups of 10 or fewer, with food and water continuously available. Flies were kept in similar groups in 150 cc wide-mouthed bottles.

Results. The detailed data are shown in Tables I and II, and are summarized and compared with similar data for DDT in Table III. It will be seen (Tables I and II) that in the region of the LD_{50}, changes in dose of γ 666 made relatively little difference in terms of mortality. This plateau-like region of the toxicity curve is thought to be due to gross inhomogeneity of the test animals. Neither sexes nor specific ages were carefully selected and random variations in

† 1, 2, 3, 4, 5, 6, hexachlorocyclohexane or "666" is a mixture of alpha, beta, gamma and delta isomers. The name "Gammexane" refers to the active gamma isomer as does "γ 666."

‡ 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane.

1 Bliss, C. L., *Ann. Appl. Biol.*, 1935, **22**, 134.

2 Bracey, P., and David, W. A. L., (British) *Insecticide Development Panel*, 1944, (44), 170.

3 Cameron, G. R., and Burgess, F., (British) *Insecticide Development Panel*, 1944, (44), 131.

4 David, W. A. L., (British) *Insecticide Development Panel*, 1945, (45), 236.

5 Gersdorff, W. A., and McGovran, E. R., *Soap and Sanitary Chemicals*, 1945, **21**, 117.

6 Jenkins, D. W., U. S. Army Service Forces, Chemical Warfare Service, Medical Division Report No. 56, Edgewood Arsenal, Md., Sept. 26, 1945.

7 Kearns, C. W., Ingle, L., and Metcalf, R. L., *J. Econ. Ent.*, 1945, **38**, 661.

8 Madden, A. H., Lindquist, A. W., and Jones, H. A., *U. S. Dept. of Agric. Bur. Ent. and Plant Quar. Int. Rep.*, No. 0-101.

9 McGovran, E. R., Gersdorff, W. A., Fales, J. H., and Piquett, P. G., (British) *Insecticide Development Panel*, 1944, (44), 209.

10 McMaster, P. D., Rockefeller Inst. for Med. Research, N. Y. C., personal communication.

11 Richards, A. G., and Cutkomp, L. K., *Biol. Bull.*, in press.

12 Slade, R., (British) *The Hurter Memorial Lecture, Insecticide Development Panel*, 1945, (45), 237.

13 Tobias, J. M., Kollros, J. J., and Savit, J., *J. Pharm. and Exp. Ther.*, 1946, **86**, 287.

14 Tobias, J. M., and Kollros, J. J., in press.

15 Tobias, J. M., Kollros, J. J., and Savit, J., in press.

TABLE I.
Toxicity of γ 666 for the Cockroach *(Periplaneta americana)*.

Route	No. roaches	Vol. given mm³	γ 666 mg per kg	% mortality at hours indicated 24	48	72	96	120
Surface	10	1.0	0	0	0	0	0	0
	20	1.0	1	10	10	15	20	20
	22	0.4-1.0	4	36	45	50	50	50
	25	0.6-1.0	6	28	52	56	56	56
	10	0.8	8	60	70	80	80	80
	49	1.0	10	18	45	49	57	59
	20	1.0-2.0	20	20	60	65	70	75
Injection	10	1.0	0	0	0	0	0	0
	10	1.0	1	0	10	10	20	20
	10	1.0	4	20	50	60	70	70
	10	1.0	6	20	20	30	30	40
	20	1.0	10	20	65	75	80	80
	10	1.0	20	30	60	70	80	80

Graphically determined
Approximate 120-hr LD_{50} (surface), 5 mg per kg (range 4 to 7.5).
Approximate 120-hr LD_{50} (injection), 4 mg per kg (range 3 to 7.5).
By method of Bliss[1]
120-hr LD_{50} (surface), 4.57 mg per kg (fiducial limits 1.0-9.4 mg per kg).
120-hr LD_{50} (injection), 3.39 mg per kg (fiducial limits less than 0.5-1.3 mg per kg).

TABLE II.
Toxicity of γ 666 Applied to the Body Surface of Flies (in Acetone).

Species	No. flies	Vol. applied mm³	γ 666 mg per kg	% mortality at hours indicated 24	48
Musca domestica	25	0.4	0.3	16	20
(newly emerged, 1-18 hr)	25	0.6	0.4	52	60
	25	0.8	0.5	56	60
	22	1.0	0.7	91	95
(older adults)	31	1.0	0	3	3
	20	1.0	0.3	5	5
	30	0.4	0.5	30	30
	30	0.6	0.8	33	53
	50	0.6	1.4	64	67
	15	1.0	1.8	87	100
	10	0.4	2.7	90	90
	5	1.0	3.5	100	—
	20	0.6	6.0	100	—
	19	1.0	7.0	100	—
Calliphora spp.	33	0.4	0.5	24	42
(older adults)	20	0.6	0.7	10	60
	32	0.8	0.9	41	65
	15	1.0	1.2	80	100

Graphically determined
Approximate 48-hr LD_{50} (*Musca*-newly emerged), 0.4 mg per kg (range 0.4 to 0.5).
Approximate 48-hr LD_{50} (*Musca*-older adults), 1 mg per kg (range 0.7 to 1.2).
Approximate 48-hr LD_{50} (*Call. spp.*-older adults), 0.6 mg per kg (range 0.6 to 0.7).
By method of Bliss
48-hr LD_{50} (*Musca*-newly emerged), 0.4 mg per kg (fiducial limits less than 0.1-0.65 mg per kg).
48-hr LD_{50} (*Musca*-older adults), 0.83 mg per kg (fiducial limits, 0.63-1.1 mg per kg).
48-hr LD_{50} (*Call. spp.*-older adults), 0.6 mg per kg (fiducial limits, less than 0.1 to more than 1.2 mg per kg).

TABLE III.
Comparative Toxicity of γ 666 and DDT for Flies and Cockroaches.*

Insect	Approximate LD_{50}, mg per kg†			
	Surface		Injection	
	γ 666	DDT	γ 666	DDT
Periplaneta americana	5	10	4	5-8‡
Musca domestica				
(newly emerged)	0.4	2	—	—
(older adults)	1	8-21	—	—
Calliphora spp.				
(older adults)	0.6	9-28	—	—

* DDT data.[13]
† Roach observation period 120 hours, fly 48. Solvent acetone in all cases.
‡ Probably closer to 8.[13]
N.B. All data in this table graphically determined.

either or both of these categories could account for such a spread.

From Tables I and III it can be seen that the LD_{50} for γ 666 applied to the surface of the cockroach (4.6 mg per kg) is not significantly different from that for intra-abdominal injection (3.4 mg per kg). This approximate equivalence in toxicity of surface-applied and of injected material is a most interesting phenomenon, and has also been demonstrated, in the case of the cockroach, for DDT.[13] It is not known to the authors to occur with any toxic agent administered to mammals. The finding again highlights the importance of the extremely efficient absorption of certain substances by the insect body surface. It is most unlikely that this absorption took place through spiracles, since in the roach, where application was easily localizable, the toxic agent was placed on the body at a considerable distance from the spiracles, and inspection revealed rapid evaporation of solvent with little flow. In the case of DDT, in addition, there has been shown to be a positive correlation between the presence of a chitinous exoskeleton and susceptibility to the external application of the toxic agent.[11]

Gamma 666 is only about twice as toxic as DDT for the cockroach (Table III). For the adult fly, however, it is distinctly more toxic (LD_{50}, 0.8 mg per kg) than DDT (LD_{50}, 8 to 28 mg per kg). The data show that it is also somewhat more toxic ($p = 0.05$) for the newly emerged fly *(Musca domestica)* (LD_{50}, 0.4 mg per kg) than for the adult (LD_{50}, 0.8 mg per kg), but the decrease in sensitivity as the fly ages is less than that reported for DDT (Table III).[13] The toxicity data reported here suggest a somewhat greater absolute toxicity of γ 666 for adult flies than do those of David[4] who reported a maximum median lethal dose of 2 and 3 mg per kg for the male and female respectively of *Musca domestica*. The difference is small, however, and may not be a meaningful one.

Those flies *(Musca domestica)* and roaches *(Periplaneta americana)* which die after a surface applied LD_{50} dose of γ 666 do so more quickly than after a comparable dose of DDT.[13] In addition, although the times have not been measured, it is the strong impression of the authors that these insects show symptoms of poisoning (tremors, ataxia, convulsions, falling, prostration) much sooner after γ 666 than after a comparably toxic dose of DDT. This is important, since a faster "knockdown rate" implies less time for ranging and oviposition.

Conclusions. 1. The approximate LD_{50} for γ 666 in the cockroach *(Periplaneta americana)* is 4.6 mg per kg when applied to the body surface and 3.4 mg per kg when injected intra-abdominally. These values are not significantly different.

2. An approximate equivalence, similar to that noted above, between surface and injection toxicity for the roach has been reported for DDT. This finding emphasizes the importance of the absorptive capacity of the insect body surface for contact poisons

in contributing to the effectiveness of insecticides.

3. The approximate LD_{50} for γ 666 applied to the body surface is 0.4 mg per kg for the newly emerged fly *(Musca domestica)*. For the older adult the LD_{50} is about 0.8 mg per kg for *Musca domestica* and 0.6 mg per kg for *Calliphora spp.* This decrease in sensitivity as the fly ages is less than that reported for DDT.

4. Gamma 666 is about twice as toxic as DDT for the cockroach *(Periplaneta americana)*, and is distinctly more toxic than DDT for *Musca domestica* (newly emerged and adult) and *Calliphora spp.* (adult).

5. Both death and knockdown occur more rapidly after surface application of γ 666 to *Musca domestica* and *Periplaneta americana* than after DDT.

15368

Type-specific Capsular Swelling of Meningococci by Chicken Antiserum.

KELSEY C. MILNER AND MORRIS F. SHAFFER.

From the Department of Pathology and Bacteriology, School of Medicine, Tulane University, New Orleans, La.

Branham[1] has recently emphasized the value of serologic typing of meningococci for epidemiologic studies as well as for more exact etiologic diagnosis. The methods currently employed for this purpose include (a) capsular swelling with hyperimmune rabbit serum and (b) agglutination with monovalent antisera prepared in rabbits or chickens. One of the advantages of using chickens as a source of agglutinating serum is their ability to tolerate doses of meningococci which may be toxic or even lethal for rabbits.[2,3]

During the past 18 months we have had occasion to prepare such antisera in adult chickens. The course of injections was based on the report of Phair, Smith and Root.[2] Doses of 2 to 4 billion living organisms, derived from casein-hydrolysate starch agar cultures[4] and suspended in physiological saline, were given intravenously at intervals of approximately one week. After 4 injections the animals were allowed a rest period of 7 to 16 days before being bled; in some instances this bleeding was followed after 7 to 9 days by a fifth injection and the animals were kept 1 to 4 weeks longer before final exsanguination. The serum collected from the successive bleedings of each chicken was pooled and stored at 2°C. Throughout the period of immunization the birds appeared healthy and showed no appreciable loss of weight.

In testing for agglutinins, meningococci from cultures grown 5 to 18 hours on casein-hydrolysate starch agar were suspended in saline to a concentration of 2 to 4 billion cocci per ml. Dilutions of antiserum were mixed with an equal volume (0.3 ml) of culture suspension in small tubes and these were agitated vigorously on a Kahn shaking machine for 10 to 20 minutes at room temperature before readings were made. Agglutination was readily visible with the naked eye. The titer of the serum was recorded as the highest dilution, after addition of antigen, in which clumping was marked. The results summarized in Table I show that following immunization of chickens with 16- to 18-hour cultures or with cultures only 4 hours old, we obtained satisfactory agglutinating sera versus strains of meningococci

[1] Branham, S. E., *Am. J. Pub. Health*, 1945, **35**, 232.

[2] Phair, J. J., Smith, D. G., and Root, C. M., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 72.

[3] Miller, C. P., *Yale J. Biol. and Med.*, 1944, **16**, 519.

[4] Mueller, J. H., and Hinton, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 330.