pounds such as acetate (*3*). The specificity of the insect requirement for sterols has been subjected to detailed investigation by a number of workers, and some of the important configurational requirements have been elucidated (*3*).

The metabolism of sterols by insects has been investigated much less intensively than have the nutritional aspects. Noland (*4*) postulated that certain nutritionally inadequate sterols could not be utilized by the insect because they inhibited the esterification necessary for assimilation from the intestinal tract. This hypothesis was not well supported by the results of a study of the structural specificity of sterol esterification *in vitro* by cockroach gut homogenates (*5*). Using larvae of *Callosobruchus chinensis* L. (cow pea weevil), Ishii (*6*) found that both tetrahydrostigmasterol and epicholestanol were nutritionally adequate for larval growth. Analyses of the sterols contained in the tissues of larvae that were fed on diets containing either of these unnatural compounds as the only dietary sterol indicated that the sterol nuclei had not been metabolically altered. Ishii concluded that the larvae were able to utilize tetrahydrostigmasterol without *in vivo* dehydrogenation at C_5 and C_6 and that they could utilize epicholestanol without isomerization of the hydroxy at C_3.

That an animal could be so loosely organized that a variety of sterols could be utilized at a cellular level without first being subjected to metabolic conversion into steroid compounds typical of the tissues seemed highly unlikely to us. A study was, therefore, undertaken of the effect of dietary sterols on tissue sterols in larvae of the confused flour beetle, *Tribolium confusum* Duval (*7*). In these experiments, the beetle larvae were reared from egg to larval maturity on synthetic diets to which known amounts of pure sterols had been added. The sterols used were cholesterol, 7-dehydrocholesterol, dihydrocholesterol, sitosterol, and ergosterol. The basal synthetic diet was composed of sterol-free fibrin, vitamin test casein, soluble starch, inorganic salts, and a mixture of ten B vitamins.

Before analysis, the larvae were held for 24 hours on a sterol-free diet to minimize interference from sterols contained in the gut contents. The larvae were then homogenized and extracted with a 1/1 mixture of acetone and absolute alcohol. Sterols were precipitated with digitonin. A modified Schoenheimer-Sperry reagent was used for color development, and absorbance after 1.5 and 33 minutes was measured at 620 mμ with a Beckman DU spectrophotometer. This procedure allows an estimation of those sterols which develop maximum color at 1.5 minutes (Δ5, 7) and of those that develop color maximum at about 33 minutes (Δ5) (*8*).

In the present study, 7-hydrocholesterol and cholesterol were used as standards for the "fast" and "slow" sterols, respectively. This analytic method does not permit estimation of sterols which either do not precipitate with digitonin or do not develop color with the Schoenheimer-Sperry reagent. In order to characterize further the "fast" and "slow" sterols found in the tissue extracts, and in order to detect sterols not detectable by the chemical method, a paper chromatographic method was employed. The method of McMahon *et al.* (*9*) was modified for this purpose. Whatman No. 1 filter paper was used, with antimony pentachloride in chloroform as the chromogenic agent. The method was standardized, employing 7-dehydrocholesterol, cholesterol, sitosterol, and dihydrocholesterol. With an ascending solvent mixture of 13 parts phenol, 30 parts methanol, and 57 parts water applied in one direction and a 14/45/41 mixture in the second direction, a two-dimensional chromatogram was obtained in which the several sterols were clearly separated.

Larvae grown on a natural diet (graham flour) and larvae grown on synthetic diets (each containing one of the pure sterols) were analyzed by both chemical and chromatographic methods. All experiments were replicated three times, and all analyses were run in duplicate. In every case, the principal tissue sterol present was a "fast" sterol which, by both its rate of color development with the Schoenheimer-Sperry reagent and its position on the paper chromatogram, was indistinguishable from 7-dehydrocholesterol. Regardless of the identity of the dietary sterol, this tissue sterol was present at concentrations between 850 and 900 μg/g of tissue. In all larval samples, a second sterol was also found, in amounts from 410 to 450 μg/g, and was chemically and chromatographically indistinguishable from cholesterol. No other tissue sterols were detected in amounts that permitted reasonably good identification. Chemical and chromatographic analyses of larvae from the different diets showed no differences attributable to the identity of the sterols in the diets.

Experiments were also set up in which beetle larvae were started on synthetic diets containing either no sterol or one of the nutritionally inadequate sterols, such as calciferol, progesterone, and testosterone. On these diets, however, no larval growth occurred, and none of the insects could be recovered and analyzed. Dihydrocholesterol diets did not promote optimum larval growth, although sufficient numbers of larvae were obtained to run the required analyses.

The results obtained in this study clearly indicate that *Tribolium confusum* larvae can metabolize dietary sterols to the extent of altering the side chain and the degree of saturation in the B ring of the nucleus. The nutritional adequacy of different steroid compounds is probably determined largely by the ability of the insect tissues to convert them into cholesterol and 7-dehydrocholesterol or compounds so closely related to these that they could not be distinguished from them by the methods employed in this study.

STANLEY D. BECK
GEETA G. KAPADIA
Department of Entomology,
University of Wisconsin, Madison

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Cytolysis versus Differentiation in Antineurula Serum

Complex antigenic mixtures derived from various developmental stages of *Rana pipiens* are being studied (*1*) in an analysis of the chemical patterns of ontogeny (*2*). Antigens were prepared from entire neurulae (Shumway stages 14 and 15) by a standardized homogenization in a Ten Broek tissue grinder with 0.05*M* phosphate-buffered saline at *p*H 7.4, and collection of the supernatants following centrifugation at 1500 *g* with final clearing at 10,000 *g* (*3*). Such supernatants, containing 1.6 to 2.0 mg of total N per milliliter, were emulsified (*4*), and quantities containing 0.2 ml of the antigenic mixture were injected under each scapula of four American chinchilla rabbits. Antisera were collected at periodic intervals and screened, using the interface precipitin reaction and the Ouchterlony agar diffusion technique (*5*). A representative antiserum, collected 4 months after injection, yielded a positive antigen dilution titer at 0.2 μg of N per milliliter. Similar titers were obtained with γ-globulin fractions prepared by a cold ethanol

Table 1. "Dorsal tissues" of *Rana pipiens* neurulae cultivated in serologic media. Data refer to numbers of cultures. Numbers in parentheses: first number, number of rabbits; second number, number of sera. The minus signs in column 2 indicate that no precipitate formed.

Sera	Condition of cultures at 7 days, 20° to 24°C				
	Precipitate	Lysis	Poor	Normal	Total
Normal rabbit serum (4–4)	−	3	27	27	57
Antineurula supernatant (4–7)	+	91	5	0	96
Normal rabbit β-globulin (2–2)	−	0	2	18	20
Antineurula supernatant β-globulin (1–2)	±	0	28	5	33
Normal rabbit γ-globulin (2–2)	−	0	2	33	35
Antineurula supernatant γ-globulin (2–3)	+	57	8	0	65
Antineurula supernatant (1–5), crude and γ-globulin, absorbed with					
Neurula supernatant	−	0	40	27	67
Neurula pseudoglobulin	+	0	49	0	49
Neurula vitellin	+	32	5	0	37

method (*6*) which was controlled by microelectrophoretic analysis (*7*).

Fragments of dorsal trunk tissues, obtained from stage-15 neurulae, which contained medullary plate, neural crest, notochord, dorsal mesoderm, and, occasionally, epidermal elements, were cultured singly in hanging drops of Niu-Twitty balanced salt medium plus 10 units of penicillin and 1 μg of streptomycin per milliliter. After 7 to 14 days, these normal cultures contained chromatophores, neuroblasts, chorda, fibroblasts, striated fibroblasts, mesenchyme, and, occasionally, ciliated epidermal cells. Similar cultures (Table 1) were made in media containing crude normal rabbit sera, crude antineurula supernatant sera, normal rabbit β- and γ-globulin, and antineurula supernatant β- and γ-globulin which had previously been dialyzed against Niu-Twitty medium. These media contained rabbit protein in amounts varying between 4.5 and 0.7 percent. Control tests at varying dilutions of these reagents indicated that the nature of the results was independent of the rabbit protein concentration per se within this range.

In Table 1, tabulations for undifferentiated but living cultures and cultures showing only epidermal and mesenchymal differentiation are reported together under the heading "poor." Some as yet unknown factors in normal rabbit sera were mildly toxic (undifferentiated, 8 cases; epidermal and mesenchymal differentiation, 19 cases). However, development in normal γ-globulin was identical with that shown in Niu-Twitty control cultures. The crude antisera and their γ-globulins were potent cytolytic agents, while their β-globulins showed some toxicity (undifferentiated, 10 cases; epidermal and mesenchymal differentiation, 18 cases). This cytolytic activity was independent of complement, which, in initial tests, was destroyed by heating at 56°C for 30 minutes. These observations on *Rana pipiens* are in close agreement with those made by one of us (*8*) on *Hynobius nigrescens,* also using antineurula supernatant in tissue cultures.

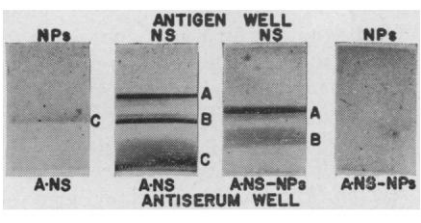

Fig. 1. Agar diffusion patterns with a frog neurula-antineurula system. NPs, neurula pseudoglobulin; NS, neurula supernatant; A.NS, antineurula supernatant; A.NS-NPs, antineurula supernatant absorbed with neurula pseudoglobulin.

In a further examination of specificity, these antisera were absorbed with a number of reagents. Among these were the water-soluble (pseudoglobulin or livetin) and water-insoluble (vitellin) proteins obtained (*3*) from repeatedly washed neurula yolk platelets.

The various line patterns (Fig. 1, *a, b,* and *c*) found in agar diffusion analyses of unabsorbed and absorbed antisera will be identified in another context; however, attention is invited to the line labeled *c*. In spite of differences in its rate of diffusion in the complex mixture and purified system, it was found to be continuous between the wells containing the homologous reagent and the pseudoglobulin in appropriately oriented plates —a condition which suggests identity (*9*). This identity is supported by the absence of the line in patterns produced by the absorbed antisera. Other types of pseudoglobulin preparations produced two lines with these antisera, one of which was identical to line *c*. This fits well with other evidence (*10*).

It may be seen in Table 1 that absorption with pseudoglobulin removed all the cytolysis observed with the unabsorbed antisera. Some level of cellular differentiation was possible (undifferentiated, 27 cases; epidermal and mesenchymal differentiation, 22 cases) even in the presence of precipitate from other serologic systems. However, this differentiation remained at a much lower level than that in the unabsorbed (normal rabbit serum: undifferentiated, 8 cases; epidermal and mesenchymal differentiation, 19 cases) or neurula supernatant absorbed (undifferentiated, 2 cases; epidermal and mesenchymal differentiation, 38 cases) controls as meastured by the variey of cell types which developed.

Antisera absorbed with vitellin retained the cytolytic activity of the unbsorbed antisera. This corresponds with earlier evidence of the serologic nonidentity of pseudoglobulin and vitellin (*3*) and must be taken into account in considerations of the functional relationships of these materials (*10, 11*). Certainly this nonidentity is dependent on more extensive molecular changes than those implied in a conversion of vitellin to pseudoglobulin by dephosphorylation alone (*12*).

These experiments suggest that the cytolytic activity of antineurula sera can be referred to one or more antigenic systems represented among the serologically active groupings on pseudoglobulin and may provide a model to explain inconsistencies in the cytolytic activity of various sera as reported in the literature (*2*). The low level of differentiation obtained after absorption with pseudoglobulin suggests that other antigenic factors (Fig. 1, *a* and *b*) are more closely related to differentiation.

It will not be known whether the cytolytic or differentiation-inhibiting activity, or both, is localized in the cell membrane or whether the antibody penetrates the cell, until fluorescent antibody studies now in progress are completed. The possibility that pseudoglobulin controls certain characteristics of the cell membrane may explain the observations on which the suggestion of its inductive role was based (*12*).

GEORGE W. NACE
KAZUKO INOUE*
Zoology Department, Duke University, Durham, North Carolina

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* On leave from the Pathology Department, Medical School, Kanazawa University, and from Kanazawa Women's College, Kanazawa, Japan.

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Thermal Protection of Choline Chloride from Decomposition by Ionizing Radiation

Changes produced by ionizing radiations in target materials—for example, polymerization of vinyl monomers (*1*) and inactivation of enzymes (*2*)—are in general enhanced by temperature elevation. It appears, however, that choline chloride, which at room temperature is one of the most radiosensitive organic solids known (*3*), becomes markedly radiation-resistant at 150°C.

Studies have been carried out at our laboratory (*4*) on the effects of ionizing radiation on nerve tissue constituents, including the cholinesterase system (*5*). Choline, a substance essential to nerve conduction, has been shown (*3*) to decompose by a free radical chain mechanism to trimethylamine and acetaldehyde when it is subjected to ionizing radiation as the pure crystalline chloride. When exposed at room temperature to 2-Mev electrons, Co^{60} γ-rays, or C^{14} β-rays, the *G* values—that is, the number of molecules decomposed per 100 ev—were 20, 175, and 1250 respectively; at −196°C the compound was stable.

In an attempt to determine the energy of activation of the radiation decomposition of choline chloride, the crystalline compound was exposed to Co^{60} γ-rays at room temperature, 50°C, and 150°C. At each temperature, three Pyrex ampules of twice recrystallized choline chloride, dried under vacuum and nitrogen at 110°C for 2 hours and vacuum sealed, were exposed to the radiation for varying periods of time. Three Co^{60} sources were used, two of which (delivering 232,000 and 792,000 rep/hr) were maintained at room temperature; the third (572,000 rep/hr) had a normal operating temperature of 50°C and was equipped with a furnace for higher temperatures. The percentage of remaining choline in each of the irradiated samples and its control was determined by the reineckate method (*6*). The *G* values, which are listed in Table 1, were then calculated for each run from the obtained semilogarithmic relationship between the percentage of remaining choline and the radiation dose in rep.

Decreasing the radiation dose rate or increasing the temperature from 20° to 50°C resulted in higher yields of decomposition. However, at 150°, regardless of the radiation exposure (4.6 to 13.7×10^6 rep) only 9 to 13 percent of the choline decomposed. The nonirradiated control, which was also kept at 150°C, did not change in appearance. The irradiated samples, however, became brown, and a small amount of insoluble material formed.

In view of these changes, it was necessary to determine whether more than one compound was responsible for the very high choline recovery as indicated by the reineckate analyses. Cholinemethyl-C^{14} chloride was synthesized (*3*) and recrystallized twice. The product had a specific activity of 66.5 mμc/mg of choline chloride (*7*) (calculated, 62.5 mμc/mg). It was shown to be chromatographically pure (Whatman No. 1 paper and 4/1/1 *n*-butanol, concentrated HCl, and water, followed by autoradiography). The labeled choline was irradiated at 150°C in the same manner as the nonlabeled material. Once again, some brown and insoluble substances were formed.

Analysis of the soluble material by the reineckate procedure indicated that the choline recoveries following exposure to 1.4, 2.8, and 3.7×10^7 rep were 94, 94, and 93 percent, respectively (*8*). Solutions of the sample which had been irradiated for 64.5 hours at 150°C, and of the labeled control, which also had been held at 150°C for the same length of time, were chromatographed for 23 hours. Included on the same chromatogram were samples of C^{14}-labeled trimethylamine and nonheated, nonirradiated, labeled choline. The heated and nonheated control samples showed a single spot only. The irradiated sample activity was predominantly at the same R_f as the choline controls with only a faint trace (about 1 percent) at a slightly higher R_f. Apparently, therefore, no C^{14}-labeled compound other than choline had contributed to the color developed in the analytic procedure.

It might be assumed that the brown, irradiation-induced materials had acted as inhibitors of the free radical chain degradation of the remaining choline. If this were the case, one would expect that this type of inhibition should have been evident not only at 150°C but also, to some degree, at lower temperatures.

Lemmon (*3*) has suggested that the spatial arrangement of atoms of crystalline choline chloride may play an important role in the free radical chain degradation. One might speculate that thermal excitation at 150°C (in contrast to that at lower temperatures or to the excitation due to ionizing radiation per se) can disturb the arrangement sufficiently to prevent the chain reaction. Further studies, therefore, may help to determine the relationship of crystalline structure to free radical chain reactions in solids, as well as to establish the use of elevated temperatures to protect some labile materials during irradiation.

Table 1. Effect of temperature and dose rate on Co^{60} γ-ray decomposition of choline.

Co^{60} source (rep/hr)	Temp. of irradiation (°C)	Dose causing 50% decompn. (reps)	G values
792,000	18–20	2.9×10^7	143
232,000	20–25	1.0×10^7	415
572,000	50	0.8×10^7	520
572,000	150	*	

* Regardless of the dose—that is, from 0.5 to 3.7×10^7 rep.—approximately only 10 percent of the choline decomposed.

I. SERLIN*

Medical Department,
Brookhaven National Laboratory,
Upton, New York

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8. Since the furnace in this Co^{60} source required approximately 15 minutes to either heat up or cool down from 150°C after the addition or removal of a sample, it is possible that the observed small choline losses were initiated in these periods.

* Present address: Shawinigan Resins Corporation, Springfield, Mass.

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Segregation of Plasmagenes and the Determination Problem

Investigations on the willow-herb (*Epilobium*) have shown that the intra-individual segregation of plasmagenes is a basic character of cytoplasmic inheritance (*1*). During vegetative cell divisions the plasmagenes may be distributed accidentally. They may, however, enter more or less exclusively one of the daughter cells as well. In such a way differences of cells and characteristic patterns arise within the plant. Besides the cytoplasmic segregation occurring in