dergo partial detoxification, while the whole venom is not affected. This may point to the presence in this venom of an additional, as yet not defined, toxic component.

Resistance of *Vipera palestinae* neurotoxin to formaldehyde treatment at low pH and the lack of antigenicity of both untreated and detoxified fraction may represent a general characteristic of snake venom neurotoxin. Also *Walterinnesia aegyptia* and *Pseudocerastes fieldii* venoms, the toxicity of which is due to neurotoxin mainly(4), are equally insusceptible to the detoxifying action of formaldehyde(10). This, together with the stability of all these neurotoxins to heat and treatment with HCl(10) raises the question of their chemical nature. The above properties of the neurotoxins of these venoms would seem to indicate that they are non-protein in nature and of low molecular weight, similarly to neurotoxins of other snake venoms(11,12, 13,14) and of scorpion venoms(15).

Summary. The effect of formaldehyde on the toxicity of *Vipera palestinae* and *Echis colorata* and their chromatographic fractions was investigated. The detoxification was found to be pH dependent. *Vipera palestinae* hemorrhagin was detoxified over a wide pH range, from 5.5 to 9.2, but its antigenicity was preserved at acidic pH only. The neurotoxin of *Vipera palestinae* was detoxified by formaldehyde at alkaline pH only. The detoxified neurotoxin was devoid of immunogenic activity. Treatment of *Echis colorata* venom with formaldehyde over a wide pH range did not result in complete detoxification although its separated hemorrhagins were detoxified.

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Effect of Deuterium Oxide on Lipid Components of L-5178Y Cells.* (28114)

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The growth characteristics of various lines of cells in tissue culture medium containing deuterium oxide have been described(1,2). Our interest has centered on the observed increase in sudanophilic material in the cells grown in D_2O. Preliminary experiments(3) carried out with cells maintained in deuterium oxide medium for 3 to 5 days indicated that the major increase in cellular lipids was due to increased glyceride content. The cells used for these analyses were in the stationary phase of growth, a time when certain strains

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of cells grown in normal medium have been shown to accumulate lipid(4,5). Earlier results were expressed as micrograms of lipid per 10^6 cells, units which did not take into account variables introduced by cell size, cell counting methods and water content of the cells. The lipid content of L-5178Y cells grown in media containing water or deuterium oxide has now been determined using cells harvested during the logarithmic phase of growth. Comparison of the lipid content (mg/g dry wt of cell) of L-5178Y cells grown in water and D_2O is the basis of this report.

Materials and methods. L-5178Y is a stable cell line derived from a murine leukemia which does not adhere to glass but can be grown in suspension as either a stationary or agitated culture(6). The cells were grown as an agitated culture in medium containing 30% of redistilled D_2O (>99.4%) since earlier work(1,2) had shown that good cell yields could be obtained at this concentration of D_2O. The medium used was a balanced salt solution supplemented with 2% horse serum(6). The horse serum used in this study was taken from a single lot in order to minimize lipid changes induced by variability in the serum lipids. Cultures were checked periodically to insure absence of contaminating pleuropneumonia-like organisms(7).

To overcome variations in growth of cells within individual flasks, cells were grown in a large number of small flasks using a standard inoculum. At 12 hour intervals, several flasks were pooled and the cells harvested by centrifugation.

The cells thus obtained were washed twice with physiological saline and transferred to lyophylization tubes with a known amount of saline. Weighed amounts of lyophylized cells were extracted continuously (Soxhlet extractor) for 5 hours with methylal-methanol 4:1 (8). The lipid extract was taken to dryness under nitrogen at 50-60°C. From 25-50 mg of the total lipid extract were subjected to chromatography on 10 g columns of activated silicic acid (Unisil, Clarkson Chemical Co., Williamsport, Pa.). The material to be chromatographed was applied to the column in hexane and various fractions eluted according to the general scheme of Horning *et al.* (9). The chromatographic scheme is presented in Table I. Hexane was redistilled prior to use. All other solvents were reagent grade and used as purchased.

TABLE I. Solvent Systems Used for Chromatography of L-5178Y Cell Lipids on Silicic Acid.

Fraction No.	Solvent	ml	Lipid eluted
1	Hexane-benzene 94:6	150	Hydrocarbons
2	Hexane-benzene 80:20	250	Sterol ester
3	Hexane-benzene 40:60	250	Triglyceride
4	Benzene	200	Sterol
5	Benzene-chloroform 25:75	250	Diglyceride
6	Chloroform	200	Monoglyceride
7	Chloroform-methanol 90:10	200	Phospholipid A
8	Chloroform-methanol 75:25	200	" B
9	Chloroform-methanol 33:67	250	" C
10	Methanol	200	—

Squalene was determined by the method of Rothblat *et al.*(10); sterols by the Mann modification(11) of the ferric chloride method of Zlatkis, Zak and Boyle(12); glycerides by the method of Van Handel and Zilversmit (13); free fatty acids by the procedure detailed by Albrink(14), and phospholipid phosphorus by the method of Fiske and Subbarow(15).

Lipid determinations were carried out on cells which had been incubated at 35°C for 12, 24 and 36 hours. The cells used as controls had been continuously passed in conventional medium; cells grown in D_2O had previously been passed daily in 30% D_2O for one week. The results of duplicate determinations at each of these times were averaged in order to obtain an average cellular lipid value over the logarithmic phase of growth.

In the isotope experiment sodium acetate-2-C^{14} was added (50 μc/liter) to cells grown in either D_2O or conventional medium. After column chromatography of the lipid extract of cells harvested at 12, 24 or 36 hours, an aliquot of each fraction was assayed for radioactivity in a Tracerlab liquid scintillation

TABLE II. Lipid Content (mg/g Dry Weight) of L-5178Y Cells Grown in Conventional or D_2O-Containing (30%) Medium.*

Cell fraction	Medium H_2O	Medium 30% D_2O	% change (D_2O *vs* H_2O)
Squalene	3.37 ±.68†	2.74 ± .83	− 19
Sterol ester‡	5.02 ±.53	12.89 ±1.94‖	+157
Free sterol	5.59 ±.25	7.12 ± .51¶	+ 27
Total "	10.60 ±.60	20.00 ±1.40**	+ 89
Triglyceride	1.80 ±.30	4.73 ±1.11¶	+163
Diglyceride	3.88 ±.53	3.47 ± .64	− 11
Monoglyceride	1.73 ±.33	1.21 ± .30	− 30
Phospholipid§ A	.35 ±.05	.30 ± .05	− 14
Phospholipid B	.57 ±.04	.61 ± .06	+ 7
Phospholipid C	1.36 ±.07	1.47 ± .07	+ 8
Total phospholipid	2.28 ±.06	2.38 ± .06	+ 4

* All values represent avg of 6 determinations.
† Standard error of the mean.
‡ Expressed as cholesterol.
§ Expressed as lipid phosphorus.
‖ $.01 > p > .001$.
¶ $p = .02$.
** $p < .001$.

counter (LSC-10) using 0.6% PPO and 0.02% dimethyl POPOP in toluene as the scintillator. The results were averaged to give an average specific activity for cells in the logarithmic phase of growth.

Results and discussion. Earlier studies(2) had demonstrated that the L-5178Y cell line will grow in medium containing up to 50% D_2O. With increasing D_2O concentration there is a decrease in number of cells per liter and an increase in generation time. In the present study we found that when grown in medium containing 30% D_2O, these cells had a generation time (26.4 hours) almost double that of cells grown in normal medium (13.5 hours).

The results of the cellular lipid determinations are presented in Table II. The significant differences in lipid content of cells grown in D_2O containing medium compared with cells grown in water are in triglyceride and sterol content. The large increase in sterol ester observed in the D_2O-grown cells is the principal reason for the increase in sterol content. In our earlier experiments(3) only the increase in glyceride content was observed, but the cells examined in the early experiments were harvested at a later stage in the growth cycle than those examined in this report. The fact that the major changes are observed in the triglyceride and sterol ester fractions suggests an effect on fatty acid rather than on sterol metabolism. In a preliminary isotope experiment in which sodium acetate-2-C^{14} was added to the growth medium the maximum difference between the D_2O-grown and H_2O-grown cells was in the greater incorporation of label into the triglycerides and sterol esters of the D_2O-grown cells (Table III). The difference was pronounced whether measured on the basis of specific activity or total radioactivity of the 2 fractions. Halevy and Geyer(16) found that HeLa and liver (Chang) cells when incubated with acetate-1-C^{14} showed maximum uptake of radioactivity in the phospholipid and neutral fat fractions.

The specific activity of the free sterol fraction of the D_2O-grown cells was slightly lower than that of the control cells while the squalene specific activity was higher in the D_2O-grown cells. These data suggest inhibition of sterol synthesis since there is an accumulation of radioactivity in the immediate sterol precursor. Liver homogenates prepared from livers of D_2O-fed mice show a reduction in cholesterogenesis(17).

The reduced specific activity of the cholesterol fraction of the D_2O-grown cells may also be explained by exchange of sterol between cells and medium. The interrelationships between synthesis of lipids and uptake of lipids from the medium by cells grown in tissue culture has been studied by several investigators. Bailey, Gey and Gey(18) dem-

TABLE III. Specific Activity of Lipid Fractions of L-5178Y Cells Grown in Conventional or D_2O-Containing (30%) Medium.

Cell fraction	Medium H_2O	Medium 30% D_2O
Squalene	3.7×10^3	7.2×10^3
Sterol ester	2.5×10^4	3.7×10^4
Free sterol	4.2×10^6	$.9 \times 10^6$
Triglyceride	4.1×10^5	6.6×10^5
Diglyceride	1.1×10^6	$.7 \times 10^6$
Monoglyceride	4.5×10^5	4.1×10^5
Phospholipid A	2.0×10^7	1.2×10^7
" B	1.6×10^7	1.1×10^7
" C	1.9×10^7	1.2×10^7

onstrated that MBIII lymphoblasts grown *in vitro* can remove cholesterol esters from the medium and return free cholesterol to the medium. Bailey(19) later showed that the type of serum used, but not its lipid composition, influenced cholesterol uptake by these cells. Sterol synthesis by mouse fibroblasts grown *in vitro* has also been shown to be independent of the lipid composition of the medium (20).

Our findings indicate that the major changes in lipid compisition of L-5178Y cells observed when D_2O (30%) is present in the medium are due to *de novo* synthesis of lipid, principally fatty acid. There may be some exchange of cholesterol between the cells and the medium. Elucidation of the mechanisms by which deuterium oxide affects the lipid content of L-5178Y cells and the role that the lipids of the medium play in these changes must await further experiments involving labeled lipids and lipid precursors.

Summary. A comparison has been made of the relative lipid content of L-5178Y cells grown in conventional medium or in the same medium containing 30% D_2O. Cells grown in D_2O medium contain more total lipid. The major increases in the lipids of the D_2O grown cells are due to increases in the levels of triglycerides and sterol esters. There are very few differences in cellular levels of any of the other lipid fractions. Preliminary isotope experiments indicate that the increased lipid content in the D_2O grown cells is due to increased synthesis of lipid.

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Isolation of a Cytomegalovirus from African Green Monkey. (28115)

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The cytomegaloviruses, also called salivary gland, submaxillary gland, and cytoplasmic inclusion disease viruses, are a group of biologically related, but generally species-specific viruses(1). Infection with these agents is characterized by intranuclear and intracytoplasmic inclusions in greatly hypertrophied cells primarily in the salivary glands, but in other organs as well.

The human cytomegaloviruses have been