

TABLE I.
Analytical data of gall bladder bile.

Spec. No.	Solid Matter %	Cholest. mg. %	Cholest. Solids %	Cholest. after saturation mg. %	Increase in Cholest. %
<i>Human Bile</i>					
608	4.95	112	2.26	133	18.7
612	15.0	364	2.42	408	9.3
613	18.0	610	3.38	800	31.0
614*	4.75	86	1.85	103	20.0
<i>Dog Bile</i>					
63	24.79	70	.28	180	157.0
64	25.02	130	.52	320	146.0
56	22.0	8	.03	134	1675.0
57	15.0	8	.05	96	1200.0
58	22.8	2	.01	288	14400.0
59	16.7	2	.01	428	21400.0
72†	21.28	2	.01	415	20750.0
73	18.92	20	.10	70	350.0
75	23.8	82	.34	208	253.0
84	16.47	44	.27	138	313.0
<i>Ox Bile</i>					
74	10.76	43	.40	124	288.0

* Mixture of 30 autopsy biles diluted with water.

† Mixture of 2 specimens.

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Amino Acids as the Only Nitrogen Source During Growth and Differentiation.

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Experiments are in progress on forms lower than mammals to determine the value of isolated amino acids for protein synthesis and to ascertain which acids if any are essential for, or facilitate the growth of organisms of different phylogenetic levels, and which are of importance in the process of differentiation. An attempt has been made to bring tadpoles of *Rana sylvatica* to metamorphosis on a diet in which the only N available was the N of isolated amino acids. Carbohydrates, fats and vitamins were supplied in the form of a "Basal Diet". The following amino acids were used in concentrations of 1% and 5%, added to the water: glycine, alanine, leucine, aspartic, glutamic, arginine, lysine, cystine, phenyl-alanine, tyrosine, tryptophane, proline, diiodo-tyrosine. Single acids and bi-acid mixtures were used, each acid being combined with every other. Furthermore, a progressive series was arranged, one acid

at a time being added to the previous mixture, until all 14 had been introduced.

The Growth Factor. Arginine was a good acid for growth, while signs of differentiation were very meagre in the arginine groups. Cystine proved to be a very good growth factor, closely following arginine. Phenyl-arginine acted very favorably on growth, especially when combined with another acid suitable for maintenance or growth. In combination with arginine or cystine, phenyl-alanine acted particularly well. Lysine also favored growth, histidine hardly so.

The Differentiation Factor. Phenyl-alanine and 2 other acids containing aromatic rings, tyrosine and tryptophane, belong in this group. A weak differentiation factor is present, *e. g.*, in phenyl alanine; the number of animals showing significant differentiation was high (50%); in tyrosine, 9 out of 14 cultures showed signs of differentiation; in tryptophane, a few animals showed advanced differentiation.

The Maintenance Factor. The maintenance and growth factors do not coincide in every case. Leucine is a very good acid for maintenance, not for growth. Alanine is fairly good for maintenance only. Arginine causes growth, maintaining the animals fairly well. Cystine is good in both respects. Histidine is extremely poor for maintenance, aspartic still more so. The maintenance factor is expressed plainly in the mortality rates. In tryptophane (differentiation acid), the first death and the majority of deaths occur early and close together; in arginine (growth acid), they occur later, during the middle period and also close together; in leucine (maintenance acid), deaths are late and scattered over a long period. The greater the number of acids in a mixture, the better the average maintenance. When several acids were given, the life spans of many animals were greatly prolonged without metamorphosis. The last animals died when nearly 10 months old.

It is possible to maintain amphibian larvae on isolated amino acids. One acid is insufficient, bi-acid mixtures are rather insufficient. The more complex combinations are sufficient approximately in ratio to the number of acids. However, even many acids are not always sufficient in the concentrations used by us. Differentiation did not advance much beyond the hind limb stage; in some animals, fore-limbs began to appear; in isolated cases indications of beginning tail resorption were noticed.

Diiodo-tyrosine brings on the usual early metamorphosis. Its influence can be modified by growth acids (such as arginine) so that metamorphoses appear later than in diiodo-tyrosine alone.