liver. As a result of this evidence it appears that under the circumstances where small amounts of material are slowly absorbed, intestinal acetylation assumes relative importance.

Summary. 1. Portal origin of blood in abdominal collateral veins of patients with portal hypertension may be demonstrated simply and rapidly by feeding fluorescein or thiocyanate and finding higher concentrations in the abdominal veins than in the antecubital veins.

2. Seven out of 12 patients with cirrhosis gave positive tests for the portal origin of abdominal collateral vein blood. Patients with acites are more likely to give positive results than those without ascites.

3. Evidence is presented for the acetylation of PABA by the human intestinal tract.

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Effect of Inhibitors on Phosphate Uptake in Excised Gills of the Mussel (*Mytilus edulis*). (17568)

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The factors limiting the rate of penetration of phosphate into the ciliated epithelium constituting the molluscan branchial tissue have been discussed.(1) The present study is a survey of the effects of some commonly-used metabolic inhibitors (cyanide, azide, sulfide, fluoride, iodoacetate, and cysteine) upon the phosphate uptake process.

Materials and methods. Excised fragments of the gills of *Mytilus edulis* L., a pelecypod mollusk, were obtained as previously described and placed in a shallow glass irrigation cell equipped with a thin cover glass.(1) Immediately above this was a counting tube of a Geiger-Müller circuit. The tissue was irrigated with an artificial sea water (ASW) containing 5 micromols of phosphate per liter, of which a minute fraction was labelled with radioactive phosphorus (P^{32}). The radioactivity of the tissue was estimated at intervals of about 10 minutes. When sufficient information had been obtained to describe the uptake of the isotope under these conditions, the flow of irrigant was stopped, and a new fluid was introduced containing, in addition to the other components mentioned, an inhibiting agent in 0.001 M concentration. Readings were then continued until sufficient information was obtained pertaining to the new conditions. At the conclusion of observations the tissue was removed from the glass cell and examined with the compound microscope to observe the ciliary activity. All experiments were performed at 15°C.

Results. Phosphate content of the gill fragment was expressed as a percentage of the tissue radioactivity at the time of addition of the inhibitor. Fig. 1 shows the results of plotting the data so adjusted. The heavy broken lines connect the averages obtained with each inhibitor. The lighter smooth curves, identical in each graph, show the expected phosphate uptake, based on 22 previous experiments,(1) in which the value of k, the phosphate saturation constant, was found to be 0.0050 $min.^{-1}$ The administration of cyanide, sulfide, or iodoacetate resulted in a phosphate uptake less than expected, while addition of azide or fluoride was without pronounced effect. An apparent stimulating effect of cysteine is shown in the last curve.

Rigorous statistical treatment of the data was impractical for several reasons, but an idea of the significance of the observed differences may be obtained by comparing the relative value of radioactivity obtained at a

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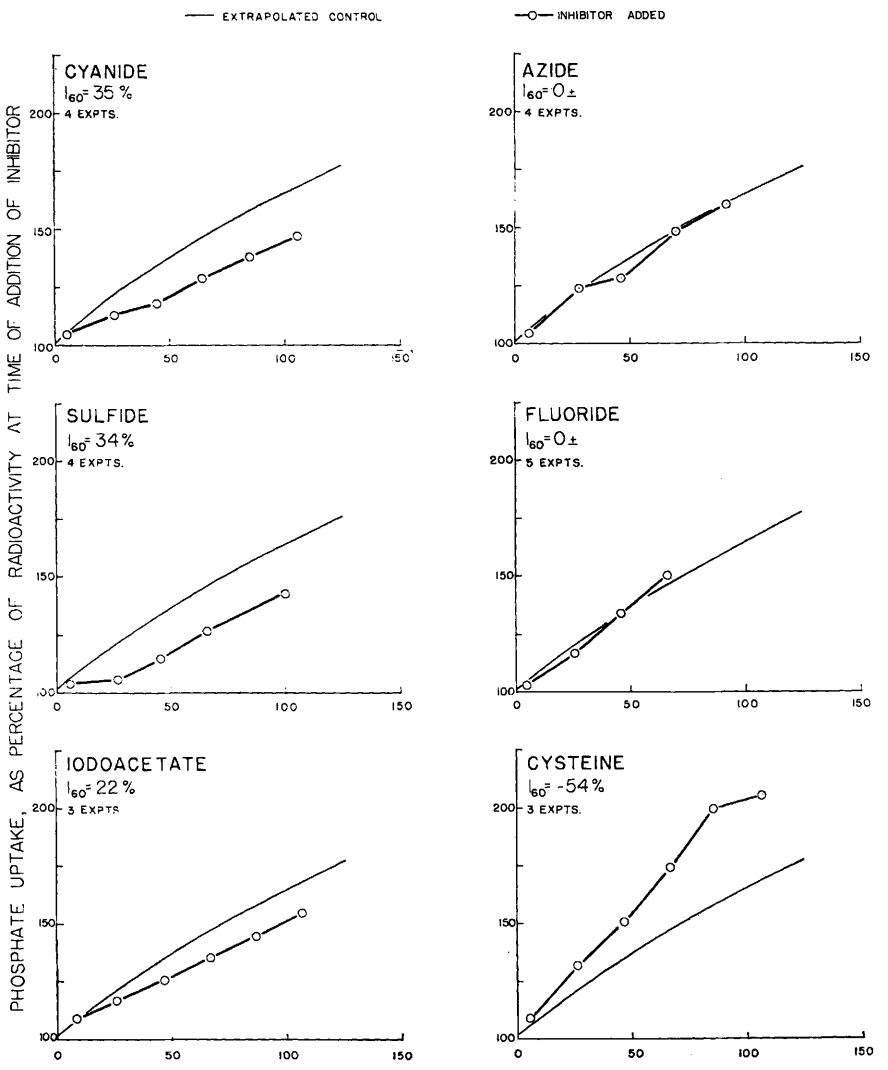

FIG. 1.

specified time after the addition of the inhibiting agent, with that at the corresponding time on the control curve. Such comparisons ["Student's" t-test(2)] were made for the observations at 60 minutes after addition of the inhibitor; the probabilities of the differences being associated with chance variation are as follows: cyanide, 0.001; sulfide, 0.001; iodoacetate, 0.05; azide, 0.5; fluoride, 0.6; cysteine, 0.014. The results of these comparisons confirm the impression that the inhibitions observed with cyanide, sulfide, and possibly iodoacetate are real effects, while the differences observed with azide and fluoride could easily arise as the result of sampling error. Microscopic examination of the gill after each experiment revealed no perceptible slowing of the ciliary beat even after 2 hours

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TABLE I.
% of Each Inhibitor Remaining Undissociated at pH 8.0 (the pH of ASW).

Inhibitor	Dissociation	Constant, as pK	Author	% undissociated
Cyanide	$HR = H^+ + R^-$	9.14	(6)	93
Azide	$HR = H^+ + R^-$	4.72	(6)	.05
Sulfide	$H_2R = H^+ + HR^-$	7.04	(6)	10
,,	$HR^- = H^+ + R^{--}$	14.92	(6)	100
Fluoride	$HR = H^+ + R^-$	3.16	(7)	.001
Iodoacetate	$HR = H^+ + R^-$	3.15	(7)	.001
Cysteine	$RCOOH = H^+ + RCOO^-$	10.28	(4)	95
,,	$RNH_2 + H_2O = RNH_3^+ + OH^-$	12.04	(4)	100
,,	$RSH = H^+ + RS^-$	8.18	(4)	60

immersion in the inhibitor.

Discussion. Two loci at which these reagents may affect the uptake of phosphate must be considered: a) the cell surface, affecting the permeability of the membrane to phosphate; and b) the cell interior, affecting the rate of turnover of organically bound phosphate, probably through selective inhibition.

In general, substances having the properties of weak acids penetrate only in the undissociated form.(3) The degree of dissociation depends on the pH and the ionic strength of the medium in which they are dissolved. Table I gives the proportion of each inhibitor in the undissociated form, using the dissociation constants available from the literature, and calculated from them by means of a suitable form of the Henderson-Hasselbalch equation.(4) Since no allowance was made for the difference between the ionic strength of ASW and that of distilled water, these figures are approximate. The table shows that cyanide, sulfide (as HS^-), and possibly cysteine, considered from this point of view, would penetrate readily and would be able to act within the cell. Iodoacetate, azide, and fluoride would penetrate scarcely at all, existing as they do almost entirely in the ionic state. Fluoride is known to penetrate cells in the ionized form, but only very slowly compared with chloride and bromide ions.(3)

Results of the present study indicate that the inhibition of phosphate uptake may take place at both loci mentioned above. Iodoacetate is unlikely to penetrate the cell rapidly; any inhibition by this reagent would suggest a general effect on the proteins at the surface of the cell. The ubiquity of glycolysis, and the observed lack of inhibition by fluoride and azide tend to confirm the nonpenetration of these strong electrolytes, and strengthen the case for possible surface action by iodoacetate.

Cyanide and sulfide, both able to penetrate the cell, were effective inhibitors. Note, however, that these affect enzyme systems which are also known to be sensitive to azide, which itself was ineffective. It is therefore suggested that cyanide and sulfide exert their inhibitory action by affecting metabolic turnover within the cell. No explanation is offered for the effect of cysteine, in the presence of which the uptake rate rose slowly. Though many systems are activated in the presence of cysteine,(5) these are usually of the proteolytic sort; it is difficult to correlate the scanty material available.

Summary. Excised fragments of gills of *Mytilus edulis* L., a pelecypod mollusk, were irrigated with artificial sea water containing P^{32}. The rate of uptake of phosphate in the presence of 0.001 M inhibitors was compared with the control rate. Inhibition of phosphate uptake in the presence of cyanide, sulfide, and iodoacetate, and the lack of inhibition by azide

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and fluoride, are considered from the point of view of available knowledge of the penetration of such substances into cells. Cyanide and sulfide, being salts of weak acids, penetrate the cells and probably affect the turnover rate of intracellular phosphate. The lack of effect with fluoride is consistent with its existence in the completely dissociated state. Iodoacetate, also completely ionized, may be effective at the cell surface, affecting the permeability of the cell to phosphate. The apparent rise in the rate of phosphate uptake in the presence of cysteine remains unexplained.

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The Sulfhydryl Content of Normal and Abnormal Human Sera.* (17569)

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The important role of the sulfhydryl group in metabolic processes has long been recognized although the precise manner in which it participates in these reactions is not clearly understood.(1,2) Kolthoff and Harris(3) have described an amperometric method for the determination of mercaptans and this technic has been adapted as a quantitative method for sulfhydryl groups in amino acids and proteins by Benesch and Benesch.(4) During the past 3 years, we have been conducting studies on the mechanism of action of various drugs and one of the determinations which has been of great interest has been that of the sulfhydryl content of the serum proteins. It was hoped that an indicator system could be developed which would aid not only in clarifying the metabolic abnormalities induced by the presence of a neoplasm but also measure the effect of chemotherapeutic agents. Although the objective of this study was to follow the progressive changes which occur in animals and patients with tumors, it also seemed desirable to investigate patients with other diseases in which changes in the serum proteins are known to occur.

Methods. Blood was obtained from patients in the fasting state and the serum carefully separated to avoid hemolysis. The fresh serum was then added to 21% methanol solution containing ammonium nitrate and ammonium hydroxide as supporting electrolytes. The current which flows between a mercury-mercuric iodide electrode and a rotating platinum electrode through the serum-methanol solution is measured with a sensitive galvanometer. As .001 N silver nitrate is added in small increments, a silver mercaptide is formed and the increment of current is small. When all the free SH groups have reacted, a slight excess of silver ion results in a relatively large increase in the diffusion current. By plotting graphically, the current flow observed against the added silver nitrate, one obtains two straight line curves whose intersection is the equivalence point.(3) The titrations are standardized with cysteine each day and the results are expressed as milligrams of cysteine per unit, *i.e.*, per 100 ml serum, gram protein nitrogen, gram albumin nitrogen, etc. It is not implied however, that the sulfhydryl determined in serum is cysteine. Proteins have been separated into albumin and globulin components by the method of Pillemer,(5) and the SH determined in each individually. Protein

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