INTRODUCTION
Studies on the geographical distribution of cancer have raised strong suspicions that a high proportion of human cancer is of environmental origin. Exposures, lasting a lifetime, to the small amounts of chemical carcinogens in the environment may rank as a major cause of human cancer. Soot was the first chemical mixture to be implicated as a carcinogen, as early as in 1775, and since then a long list of chemicals, of industrial, medicinal and metabolic origin, have been added to the list. The isolation of the carcinogenic factor, 3,4-benzo pyrene, in coal tar by Cook and co-workers (1933) triggered off an intensive search for more chemical carcinogens. 3-methylcholanthrene was synthesized separately by Wieland and Dane (1933) and Cook and Haslewood (1934). The latter group of workers also demonstrated that 3-methylcholanthrene is capable of producing neoplasms in mice. That the compound is one of the most potent carcinogens known has been repeatedly verified by various workers, whose results showed 3-methylcholanthrene to be capable of inducing neoplasms both at the site of application and at sites remote from the point of application.

Whatever might be the aetiological factor, gross imbalances in the normal metabolic processes are
associated with neoplasms. Potent carcinogens like 3-methylcholanthrene bring about a notable change in total iron (Carruthers and Suntzeff - 1942), total lipids (Wicks and Suntzeff - 1942), and in total calcium (Suntzeff and Carruthers - 1943). Though not detected earlier, Conney and Burns (1959) later observed that by six days after administration of a single dose of 3-methylcholanthrene to rats, the urinary excretion of ascorbic acid was fifty to seventy-five times greater than the control value. Several other drugs are also known to stimulate the excretion of ascorbic acid, but in none is the effect as prolonged or potent as that observed for the carcinogen. Studies by Conney and the Millers (1956, 1957) and others revealed that the injection of small amounts of polycyclic aromatic hydrocarbons, such as 3-methylcholan-
threne, to rats, rapidly induced several-fold increase
ses in the activities of certain liver microsomal enzyme systems. The work of Evans et al. (1960) has demonstrated that the enhancement of ascorbic acid excretion by drugs involves an increased rate of formation of the vitamin and its precursors, D-glucu-
ronate and L-gulomate. However, the suggestion that the chemical might influence the glucuronic acid cycle has not yet received experimental verification,
and studies on ascorbic acid-synthesizing systems failed to show the occurrence of any significant differences (Touster and Hollmann, 1961). Till today the mechanism by which 3-methylcholanthrene stimulates the metabolism of ascorbic acid is not known.

Burns et al. (1960) observed that the increase in the formation of L-ascorbic acid could be considered to result from increased metabolism of glucose through the glucuronic acid pathway. Evidence for this comes from the observation that drugs which increase the urinary excretion of ascorbic acid, also stimulate the conversion of D-glucose-1\(^{14}\)C to labelled D-glucuronic, L-gulonic and L-ascorbic acids (Longenecker et al., 1940; Horowitz and King, 1953; Burns et al. 1957).

Carbohydrates supply most of the energy required for the various activities of the tissues. The metabolism of carbohydrates includes a great network of interconnected reactions, the most important of which are shown in fig. 1. Ascorbic acid may be considered an intermediate in carbohydrate metabolism in animals according to the following pathway proposed by Burns (1960):
Fig. 1: Major pathways of carbohydrate metabolism.
- Glycolytic cycle
- Pentose phosphate pathway
- Glucuronic acid cycle
- Glycogen cycle
- Tricarboxylic acid cycle
Carbon-6-labelled L-ascorbic acid, dehydroascorbic acid and diketogulonic acid were found to be converted to glycogen, in which the label was found mainly in C-1 and C-6 of the glucose residues (Dayton et al., 1959). L-gulonolactone was found to yield glycogen labelled mainly at C-1 and C-3 (Burns et al., 1957a); labelled D-glucuronolactone was also found to be converted to liver glycogen (Eisenberg et al., 1959). These data are in agreement with the suggestion that L-gulonolactone and D-glucuronolactone are converted to glycogen through the glucuronic acid pathway, while an alternative pathway must exist for the transformation from L-ascorbic acid. Burns (1960) suggests the intermediate formation of trioses in the conversion of L-ascorbic acid to glycogen.
It has been established that deprivation of ascorbic acid results in disturbances of carbohydrate metabolism. Ganguli and Banerjee (1961) postulated that the oxidation of glucose via the Embden-Meyerhof-Parnas pathway and the tricarboxylic acid cycle is probably diminished as a result of scurvy. Banerjee (1943) observed that deficiency of vitamin C in guinea pigs leads to a change in carbohydrate metabolism as judged by i) the glycosuria ii) the diabetic type of the glucose tolerance curve and iii) the depletion of the glycogen content of the liver. Sarkar and Goswami (1975) suggested that ascorbic acid action in glycogenolysis might be through gamma-amylosis.

One of the most outstanding biochemical features of neoplastic tissues is a high rate of aerobic and anaerobic glycolysis. Warburg (1930) considered that the disturbance in glycolysis lead to tumours, while Rondoni (1955) considered the glycolytic type of mechanism to be a result rather than a cause of malignancy. Spain and Griffin (1956) observed that the cells of the central zone of the liver lost their capacity to retain or synthesize glycogen, in animals fed carcinogenic compounds. In the normal rat liver the substrate for glycolysis is glycogen, while in the neoplastic organ it is glucose (Orr and Stickland, 1941).
The growth of cancer tissue, once it has been initiated, is dependent on the availability of amino acids, and on the energy necessary for the synthesis of amino acids into the proteins of malignant cells. The amino acids must be furnished by the host. The mass flow of amino acid nitrogen quantitatively takes place chiefly within and among a restricted number of organs or tissues (fig. 2.). Cachexia is one of the most striking effects of malignant disease and is probably the most common single cause of death by cancer. In malignancy the tumour accumulates nitrogen while the host carcass loses nitrogen especially from the skeletal muscle. The tumour successfully competes with the host for the available nitrogen pool, thereby acting as a "nitrogen trap" (Mider, 1951). Rouser (1957) concluded that in a given species at a particular stage of development each normal tissue has a distribution of ninhydrin reactive constituents which is characteristic of that tissue. This characteristic pattern of the free amino acids in blood and other body fluids has been found to be altered in leukemic and breast carcinoma patients (Rouser, 1957), in tumour-bearing rats (White et al., 1954), after administration of amino acids (Christensen et al., 1948), and in menstrual cycle, pregnancy and endrocines (Soupart,
Fig. 2: Regulation of overall amino acid metabolism, from (Schepartz, 1973).
1962). Since ascorbic acid is known to be involved in the metabolism of certain amino acids and steroids, it might influence the free amino acid pattern. Cahill (1970) suggested that amino acids from skeletal muscle were released into the blood during cancer, from where they entered the liver to serve as precursors for glucose.

The level of ascorbic acid is reported to be lowered in the plasma of cancer patients (Bodansky, 1951), and in tissues of tumour-bearing rats and mice (Sure et al., 1939a); ascorbic acid has been advocated as an anticarcinogen by some workers (Schlegel et al., 1969; Warren, 1943). This view has, however, been disputed.

The present investigation was therefore undertaken to further study the effects of 3-methylcholanthrene on ascorbic acid metabolism, along the following lines:

i) The effects of 3-methylcholanthrene and ascorbic acid, separately and in combination, were studied on

a) tissue concentration and distribution of ascorbic acid

b) enzyme of glucose metabolism
c) glycogen concentration in liver

d) concentration and pattern of free amino acids in serum

ii) The effect of cancerous disease in human subjects was studied with reference to

a) blood ascorbic acid levels and urinary excretion of ascorbic acid

b) concentration and pattern of free amino acids in serum.