Metabolic Changes in Response to Acute Cerebral Ischemia Following Bilateral Carotid Artery Ligation in Arteriosclerotic Versus Nonarteriosclerotic Rats

BY BERNARD C. WEXLER, PH.D.

Abstract: Metabolic Changes in Response to Acute Cerebral Ischemia Following Bilateral Carotid Artery Ligation in Arteriosclerotic Versus Nonarteriosclerotic Rats

Both the left and right carotid arteries of arteriosclerotic and nonarteriosclerotic male and female rats were surgically ligated to induce a state of sudden and severe cerebral ischemia. The animals promptly developed signs of cerebral impairment and were sacrificed 2, 4, 6, 8, 10, 12, 24 and 48 hours later. A similar group of animals was subjected to the same regimen of carotid artery ligation except that a week's time elapsed between the ligation of each carotid artery.

As with unilateral carotid artery ligation, the bilaterally ligated animals manifested fatty metamorphosis of the liver, myocardial infarction and cerebral edema and necrosis, but the severity of these pathological changes was greatly exacerbated with the more severe ischemia.

Serum creatine phosphokinase, glutamic oxalacetic transaminase, triglycerides, free fatty acids, cholesterol, glucose and corticosterone all rose dramatically and in concert with the pathological changes, e.g., fatty liver. Animals with preexisting arterial disease exhibited much greater excursions of these pathophysiological parameters during the cerebral ischemia than did animals with normal arteries. The entire response pattern indicates that cerebral ischemia is a severe stress and is capable of eliciting highly characteristic pathophysiological changes which could be used to assess the severity or course of cerebral ischemia.

ADDITIONAL KEY WORDS arteriosclerotic breeder rats bilateral carotid ligation cerebral ischemia enzymes lipids

In a previous publication in this journal we reported finding dynamic increases in serum creatine phosphokinase, glutamic oxalacetic transaminase, triglycerides, free fatty acids and cholesterol concomitant with intense lipid mobilization and fatty infiltration of the liver, all following unilateral ligation of the carotid artery. In addition to marked hyperglycemia these animals also produced extra quantities of corticosterone. These investigations indicated that the acute cerebral ischemia of unilateral carotid artery ligation constituted a severe stress and could elicit dynamic alterations in several serum parameters, e.g.,

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enzymes, lipids, glucose and adrenocortical hormones, and the degree of change in these serum parameters was commensurate with the severity of the cerebral ischemia. Those animals having preexisting arteriosclerosis manifested the most dramatic responses to the acute carotid artery occlusion.¹

As an extension of these earlier investigations we elected to ligate both the right and left carotid arteries of male and female arteriosclerotic and nonarteriosclerotic rats to determine whether this more extensive, acute cerebral ischemia would be reflected in concomitantly more extensive excursion of the pertinent serum parameters, e.g., enzymes, lipids, glucose and adrenal steroids.

**Methods**

As many as 2,000 male and female, virgin and breeder Sprague-Dawley rats were used in order to fulfill the protocol and timed-interval samplings required by this experiment. Approximately 50% of the animals succumbed promptly to the duress of the single-stage bilateral carotid artery occlusion. Breeder rats were used because arteriosclerosis, hypertension, diabetes and other degenerative changes develop in them spontaneously.²⁻⁷ Male breeder rats develop microscopic lesions of the aorta,² coronary⁴ and carotid arteries,³ but they often succumb due to complications precipitated by vascular disease, e.g., myocardial infarction.⁸ The female breeders survive significantly longer than male breeders despite the fact that they exhibit severe, calcific, grossly visible arteriosclerosis of the aorta and kinked and tortuous carotid arteries.²⁻⁸ Virgin rats, male and female, comparable in age to the breeder rats were used as subjects free of arterial disease. The experimental format described in our earlier report¹ was followed with a few modifications.

Twenty-four animals of each of the four basic types of animals described previously were sacrificed at the outset of the experiment to provide baseline levels of all the parameters measured, i.e., males versus females, arteriosclerotic versus nonarteriosclerotic subjects. A sufficient number of extra animals were provided to ensure survival of a minimum of 24 for each time interval being considered (see below). The animals were anesthetized with secobarbital (Secodal). The same surgical procedure described earlier¹ was followed except that both carotid arteries were ligated in single-stage operation. Most of the animals which succumbed died within 15 to 30 minutes of bilateral ligation after having convulsed violently and having manifested extensor rigidity of all extremities. The surviving subjects were provided food and drink *ad libitum* and were housed in our Research Animal Colony in which light, air, temperature and humidity are carefully controlled and monitored.

In a later experiment, we wished to test whether postponement of a second ligation, by seven days, of the remaining carotid artery would reduce the severity of acute cerebral ischemia and whether it would be so reflected in the various serum parameters being measured. That is, we wished to simulate, experimentally, the clinical situation wherein cerebral ischemia is of major degree but occurs on a protracted basis rather than as a single event, i.e., precipitous bilateral occlusion versus gradual or staged bilateral occlusion. Also, because of our use of subjects with no or preexisting arteriosclerosis we wished to determine whether the seven-day interim between ligation of the right carotid artery and the left carotid artery would afford the growth of collaterals and whether this difference in extravascularization would be reflected in the nature of those serum changes which have been reliable indices of the severity of cerebral ischemia in our experience to date. Accordingly, in a second experiment, patterned closely after the first, we ligated the left carotid artery in a one-stage operation. Male and female, nonarteriosclerotic and arteriosclerotic animals were used. After seven days of convalescence the right carotid artery was ligated. Under the circumstances of single-stage carotid artery ligation, the mortality rate was much less. However, for the sake of economy, the animals were sacrificed only at those time intervals which corresponded to those times when the various serum parameters manifested their major changes following acute bilateral carotid artery occlusion, e.g., at 6, 12, 24 and 48 hours after the second carotid artery was ligated. A minimum of 24 subjects were used for each time interval for each class of animal, i.e., male and female, nonarteriosclerotic and arteriosclerotic.

The animals were sacrificed by decapitation to avoid the stress of anesthesia; blood was spun in a refrigerated centrifuge and the serum was frozen and stored until time of analysis. The same automated techniques (Auto-analyzer: Technicon) used in our previous report¹ were used. The following parameters were measured: creatine phosphokinase (CPK), glutamic oxalacetic transaminase (SGOT), triglycerides, free fatty acids, total cholesterol and glucose. In addition, serum corticosterone (Cmpd. B) levels, the main adrenocortical steroid in the rat, were also measured.⁹

At autopsy each animal was carefully examined for any evidence of cerebral or cardiovascular disease, e.g., arteriosclerosis. The
sites of carotid artery ligation were also carefully checked; animals showing questionable ligation were discarded. Pertinent tissues were saved for histopathological analyses using the same stains and methods described previously.¹

The biochemical data were subjected to statistical analyses using the Analysis of Variance method or “Students” t test prescribed by Snedecor.¹⁰ P values greater than 0.05 were considered to be not significant.

Results

General Observations

Earlier, we found that immediately following unilateral carotid artery ligation Horner’s syndrome, convulsions and extensor rigidity promptly developed in our animals.¹ Therefore, in this experiment, we were obliged to use a much greater depth of anesthesia to forestall the development of the convulsions and other untoward changes due to the greater stress of cerebral ischemia induced by bilateral ligation. Many animals succumbed soon after the second carotid artery was ligated. However, as the animals began to recover from the anesthesia they promptly showed severe blanching of both eyes, many manifested violent convulsions and died, many became paraplegic, and practically all showed marked extensor rigidity.

As in our previous report,¹ temporary fatty steatosis of the liver developed in all the animals (fig. 1). Twenty-one percent of the arteriosclerotic male breeder rats exhibited grossly visible myocardial infarction; none of these arteriosclerotic males showed any gross evidence of their arterial disease. At autopsy, 83% of the female breeders displayed grossly visible arteriosclerosis ranging from minimal to

Figure 1

Foci of cerebral necrosis induced in an arteriosclerotic female breeder rat 24 hours after bilateral carotid artery occlusion. H&E, × 50.
a much greater exacerbation of the pathophysiological changes than that seen after unilateral carotid artery ligation,\textsuperscript{1} as described subsequently.

**PATHOPHYSIOLOGICAL CHANGES IN THE SERUM Enzymes**

*Creatine Phosphokinase (CPK).* Within two to four hours after ligation of the second carotid artery there was a most dramatic increase in the serum CPK levels of all subjects and especially in the case of those having preexisting arteriosclerosis (fig. 3). The female breeders, which have the most severe arteriosclerosis, showed a significantly greater increase in serum CPK than the male subjects with less severe arteriosclerosis (fig. 3). These greatly elevated levels promptly fell toward normal levels but remained definitely elevated throughout the 48-hour course of this experiment. The nonarteriosclerotic subjects also showed a prompt and definite increase in their serum CPK levels but in no manner comparable to the arteriosclerotic subjects (fig. 3). Those animals which were permitted to convalesce for one week after unilateral carotid artery ligation and then were tested at various intervals immediately after the remaining carotid artery was ligated showed some increase, but less overt, of their serum CPK levels (fig. 3).

*Glutamic Oxalacetic Transaminase (SGOT).* All the animals, regardless of the presence or absence of preexisting arterial disease, manifested prompt and profound increases in their serum SGOT levels without any evidence of arteriosclerosis.
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FIGURE 2

Extensive fatty metamorphosis of the liver within eight hours of bilateral carotid artery occlusion. In one area (right side of photo) the greatly distended hepatic cells are filled with lipid giving each cell a "foamy" appearance. In other areas (left side of photo) the lipid appears as single, large droplets or it has coalesced into isogenous clusters of droplets. H&E, X 50.

severe degree. Many of their carotid arteries were kinked and tortuous with calcific complications. None of the nonarteriosclerotic virgin control rats showed any signs of cardiovascular disease. Unlike our experience with unilateral carotid artery ligation, practically all the animals subjected to bilateral occlusion exhibited some degree of grossly visible cerebral edema, ischemia, hemorrhage and necrosis (fig. 2).

MICROSCOPIC OBSERVATIONS

As in all our past experience, although male breeder rats do not manifest grossly visible arterial lesions they do have definite microscopic lesions consisting of intimal ground substance degenerative changes and scar tissue deposition. Similarly, the female breeder rats displayed their usual arterial lesions consisting of intimal mucopolysaccharide and collagen accumulation and medial elastolytic and calcific complications. The nonarteriosclerotic virgin control rats showed no evidence of any arterial disease (microscopic).

Again, as in our previous experiments, the nonarteriosclerotic subjects exhibited extensive atheromatous intimal growth proximal and distal to the site of ligation in each carotid artery. Further, the arteriosclerotic subjects manifested the same pattern of thrombosis superimposed upon the endothelial proliferation as described earlier. The thromboses were localized to the site of ligature injury. Therefore, it is felt that the changes described later are due to the acute cerebral ischemia induced by bilateral carotid artery ligation (surgical) rather than to the incidental and localized carotid artery thromboses. The stress of acute cerebral ischemia induced by bilateral surgical ligation of the carotid arteries caused...
Changes in serum triglyceride levels.

Triglycerides. As in the case of serum enzymes, the serum lipids showed a very prompt response to the acute cerebral ischemia. Within two hours serum triglyceride levels were significantly above normal (fig. 5). Interestingly, the nonarteriosclerotic animals exhibited a delayed peaking in their progressively elevated serum triglyceride levels, i.e., peak levels were attained at 8 to 10 hours for males and at 24 hours for females (fig. 5). None of the triglyceride levels had returned to the normal level by the close of the experiment (fig. 5). Again, although animals subjected to the regimen of delayed bilateral carotid artery ligation showed a definite increase in their triglyceride levels, these increases were not as marked as that elicited after the acute cerebral ischemia (fig. 5).

Free Fatty Acids. A rather bizarre response was recorded in the case of serum free fatty acids. The male subjects showed a definite increase in free fatty acids, but the pattern of change followed a rather erratic course (fig. 6). The female subjects exhibited a definite fall in this serum constituent but, after a similarly erratic course, an eventual peaking. Unlike the other parameters described, the serum free fatty acids were restored to normal levels. In direct contrast to the changes observed for the previous parameters, the free fatty acids were most markedly elevated in the case of those animals which were allowed to recuperate between left and right carotid artery ligations (fig. 6).

Total Cholesterol. The pattern of serum cholesterol changes was very much like the other constituents, i.e., a prompt and significant rise within a few hours after the second carotid artery was ligated (fig. 7). Unlike the
other parameters measured, the nonarteriosclerotic subjects showed the greatest increase in serum cholesterol. All subjects showed persistently elevated levels of cholesterol 48 hours after acute cerebral ischemia (fig. 7). (Due to an inadvertent technical error, the data pertaining to serum cholesterol changes for animals subjected to the two-stage carotid artery ligation became suspect and are therefore not included.)

**Carbohydrate**

**Glucose.** Very definite and prompt hyperglycemia was elicited in all animals following the acute cerebral ischemia induced by bilateral ligation (fig. 8). Most of the animals remained hyperglycemic with the notable exception of the arteriosclerotic female breeders (fig. 8). Animals permitted to recuperate from the one-sided tie-off before the second tie-off manifested the same degree of hyperglycemia as the other subjects with the more acute cerebral ischemia (fig. 8).

**Adrenal Steroids**

**Corticosterone (Cmpd. B).** All the animals, arteriosclerotic and nonarteriosclerotic, responded promptly to the stress of bilateral carotid artery occlusion as exhibited by substantial increases in the circulating levels of Cmpd. B (fig. 9). The most striking increase was manifested in the case of the arteriosclerotic female breeders which characteristically have "exhausted" adrenal glands; nonetheless, they were able to respond to the stress of bilateral carotid artery occlusion with by far the greatest increase in circulating Cmpd. B levels (fig. 9). It should be noted that these arteriosclerotic subjects continued to produce supernormal levels of Cmpd. B. Otherwise, the duration of the stress of the ischemia was acute, insofar as adrenal steroid production is concerned, since all adrenal steroid levels, with the exception of the arteriosclerotic female breeders, had returned to normal 12 to 24 hours after the onset of ischemia (fig. 9). This pattern of acute increased production of
ADRENOCORTICOSTEROIDS following acute cerebral ischemia was very much the same in those animals subjected to the interrupted carotid ligation. However, some subjects manifested unusually subnormal serum levels of Cmpd. B 12 to 48 hours after the initial duress of ischemia (fig. 9).

Discussion
These experimental investigations demonstrate that cerebral ischemia is a severe stress and will evoke pathophysiological responses, the severity of which may be used as an index of the degree of cerebral ischemia. That is, changes in such serum constituents as enzymes, glucose, lipids and adrenal steroids reflect the degree of cerebral ischemia just as changes in these same constituents may be used to reflect the degree of myocardial ischemia. Further, animals with preexisting arterial disease, e.g., kinked and tortuous calcified carotid arteries, show much greater excursion of these serum constituents following carotid artery ligation, presumably because their vertebral arteries are also sclerosed and they lack collateral arteries to supply the brain with adequate blood.

It is of interest that fatty infiltration of the liver appears to be part of the pathophysiological response to the stress of cerebral ischemia after both unilateral and bilateral carotid artery ligation. Particularly pertinent is the observation that the severity of the fatty metamorphosis of the liver is much more intense after bilateral carotid artery occlusion. For example, the distribution of hepatic lipid is much more ubiquitous following the more severe cerebral ischemia. Also, the lipid droplets appear in isogenous groups and the individual hepatocytes contain foamy, lipoidal cytoplasm—all attesting to a much more severe fatty infiltration of the liver. We believe that this steatosis of the liver is a concomitant of severe stress since we have also observed this same transitory fatty infiltration of the liver following experimentally induced myocardial infarction. It would appear that under the duress of cerebral ischemia fat is mobilized from peripheral adipose tissue sites to the liver where it is metabolized into glucose or other lipid constituents, e.g., triglycerides. If excessive quantities of hepatic triglyceride accumulate, the available lipoprotein-carrying agents may be inadequate to clear the excess accumulated triglyceride, giving rise to the temporary fatty metamorphosis of the liver. What relevance this may have to the energy requirements of the brain during times of ischemia is intriguing. Certainly, the increased production of Cmpd. B could explain the great increase in cholesterol, triglyceride and free fatty acids also observed in these animals by virtue of the potent lipid-mobilizing effects of the adrenal steroids.

Unilateral carotid artery ligation caused only minor fluctuations in serum CPK levels during the first 12 hours postligation, but CPK levels increased significantly at 24 hours and returned to normal after 48 hours had elapsed. This delayed peaking of CPK levels was construed to indicate that real cerebral damage did not occur in these animals until 24 hours after the induction of the relatively mild cerebral ischemia. However, the very prompt and significant increase in CPK levels following bilateral carotid artery ligation attests to the more severe ischemia induced by this surgical maneuver. The more dramatic increase in CPK levels of the arteriosclerotic subjects and the more prolonged increase in CPK levels would also attest to the greater degree of ischemia induced in such subjects.

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This was most likely due to the fact that these animals probably had disseminated arterial disease extending into their vertebral arteries. That is, with the major carotid arteries occluded no avenue of collateral flow to the brain was readily available because of the generalized arteriosclerosis. Similarly, the persistently elevated serum CPK levels may also be indicative of ongoing cerebral damage due to the devastating nature of the carotid ligation in subjects with preexisting arteriosclerosis. For example, the female breeders with the most severely compromised and arteriosclerotic carotid arteries exhibited the greatest excursion of CPK levels. Subjects permitted a week’s time between carotid artery ligations appeared to have affected good collateral vascular routes to the brain since their serum CPK levels were relatively only slightly elevated.

The progressive increase in serum SGOT levels previously observed to occur following the relatively moderate ischemia of unilateral carotid artery ligation was considerable. However, it in no way approached the severity and dynamic rise recorded following bilateral ligation. The increase in this enzyme, in all subjects, is most likely a reflection of the intense hepatic lipid mobilization and damage observed in these animals. Again, the persistently elevated levels of SGOT attests to the ongoing nature of the damage induced by this degree of cerebral ischemia.

In addition to the great excursions in serum enzymes the serum lipids also reflected the stressful nature of the induced cerebral ischemia. The mobilization and elevation of triglycerides observed here have been ascribed to the increased levels of circulating adrenal steroids. However, increased catecholamine release attendant upon cerebral necrosis could also account for these changes. Adrenal steroids and catecholamines have a conjoint effect upon lipid mobilization, and the changes observed here are probably due to the release of both steroids and catecholamines. We (unpublished observations) and others have observed increased adrenal steroid production to Cushing’s disease levels, increased catecholamine production and hypertriglyceridemia in patients with cerebrovascular accidents and myocardial infarction.

The bizarre oscillations in the free fatty acid levels are of particular interest. The unexpected initial drop in free fatty acid levels in the female subjects would suggest that there is a dichotomous metabolic mechanism operative in male and female rats during the stress of cerebral ischemia. After unilateral carotid artery ligation, female rats also showed a prompt and decisive drop in serum triglyceride levels. Because free fatty acids are water insoluble they must be carried into the bloodstream by albumin. The drop in free fatty acids in female rats is due either to a temporary lack in carrier albumin or to a temporary decrease in fat mobilization from adipose tissue sites—in the female only. Even more intriguing is the converse situation of supernormal levels of free fatty acids in animals subjected to interrupted carotid artery ligation. Apparently, these particular animals had an extremely efficient free fatty acid-mobilizing mechanism in operation. If true, this is provocative but difficult to reconcile in view of the relatively low levels of triglyceride in these same subjects, since one would expect a concomitant increase in circulating free fatty acids along with the triglycerides.

We were surprised to find elevated serum cholesterol levels after unilateral carotid artery ligation and even more substantial increases in cholesterol after the more severe cerebral ischemia of bilateral ligation. In our experience with patients (unpublished observations) and experimentally induced myocardial infarction we found that it is the serum triglyceride levels which best reflect this kind of vascular stress, whereas the serum cholesterol levels remain relatively unaltered.

The prompt hyperglycemia which follows acute cerebral ischemia is essentially of equal intensity after unilateral and bilateral carotid artery occlusion. However, it should be noted that the intensity of the response, in either case, is maximal.

Similarly, the intensity of Cmpd. B production is of equal magnitude following unilateral and bilateral carotid artery occlusion. Again, insofar as rodent steroidogenic responsiveness to stress is concerned, these steroid responses reported by us following cerebral ischemia are definitely maximal. That is, cerebral ischemia of any substantial degree is a considerable stressful stimulus to the hypothalamic-pituitary-adrenal axis. Of particular note is our consistent finding that female breeder rats, in addition to their severe...
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arteriosclerosis, also have lipid-depleted and hemorrhagic adrenal glands which are almost devoid of steroidogenic capacity, i.e., refractory to ACTH and enzymatically unable to convert adrenal cholesterol precursor into definitive steroids.\textsuperscript{15-17} Nonetheless, these animals are able to respond very effectively to the stress of cerebral ischemia. Of further import is the additional and consistent observation that despite their prior adrenocortical refractoriness, these arteriosclerotic female breeders not only respond most dramatically to the stress of cerebral ischemia but also they, alone, maintain persistently elevated, supernormal levels of Cmpd. B\textsuperscript{1} throughout the course of the cerebral ischemia. This is further confirmation of the stressful nature of cerebral ischemia especially in subjects which have generalized, advanced arterial disease.

In conclusion, these experimental studies demonstrate that acute cerebral ischemia induced by bilateral carotid artery occlusion virtually doubles the stressful nature of unilateral carotid artery ligation,\textsuperscript{1} and that protracted occlusion of both carotid arteries by staged ligation causes less duress than immediate bilateral occlusion. Animals with preexisting arterial disease, i.e., before induction of cerebral ischemia, manifest definite exacerbation of the untoward effects of cerebral ischemia, presumably because they are unable to affect adequate collateral blood flow to satisfy the urgent and unrelenting metabolic demands of the brain. Finally, cerebral ischemia elicits a highly characteristic pathophysiological spectrum of events ranging from serum enzyme changes, e.g., CPK and SGOT, to hyperlipidemia, e.g., triglycerides, free fatty acids and cholesterol, hyperglycemia, e.g., glucose and hyperadrenocorticism, e.g., corticosterone, which can be used to approximate the severity and course of the ischemia.

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